Review of Compounds Affecting the Biosynthesis or Bioregulation of Aflatoxins

LAURA L. ZAIKA* and ROBERT L. BUCHANAN

Microbial Food Safety Research Unit, Eastern Regional Research Center, U.S. Department of Agriculture, 600 East Mermaid Lane, Philadelphia, Pennsylvania 19118

(Received for publication November 4, 1986)

ABSTRACT

We have attempted to review the literature dealing with compounds that have been tested for their ability to inhibit growth and/or aflatoxin production by *Aspergillus flavus* and *A. parasiticus*. Although the list presented is by no means exhaustive, it serves as an indication of the type of research that has been carried out to date. A number of compounds and substances have been found that effectively inhibit fungal growth and aflatoxin production, while others have stimulatory properties. Unfortunately, most of these assessments have only dealt with fungal growth and/or aflatoxin production, and relatively few studies have attempted to identify possible mechanisms of action. Further research into the means by which other compounds influence aflatoxin synthesis is warranted and potentially could be highly beneficial to expand our understanding of mycotoxigenesis. Such studies are likely to yield knowledge that would lead to isolation of additional intermediate compounds of the pathway leading to aflatoxins, identification of key bioregulatory loci controlling aflatoxin synthesis and development of basic knowledge that would provide insights into new strategies for controlling aflatoxin formation in foods and feeds.

HISTORICAL

Aflatoxins are a group of secondary metabolites produced by the molds *Aspergillus flavus* and *Aspergillus parasiticus*. Little was known about these highly toxic and highly carcinogenic compounds before the outbreak of the "Turkey X disease" in 1960 in England. The outbreak was of such major proportion that it stimulated intensive research throughout the world.

The cause of the poultry deaths was shown to be dietary in nature and associated with a Brazilian peanut meal used as a common ingredient in the feed *(35)*. A toxic component isolated from chloroform extracts of the meal induced the disease in ducklings *(3)*, and this response was subsequently used as the basis for a bioassay for the toxin *(9)*. In 1961 Sargeant et al. *(166)*, described a paper-chromatographic technique for detection of the toxin based on a blue-fluorescing spot under UV light. Various commodities were examined and the toxin was reported to be present in peanuts from many sources. Cultures of *A. flavus* Link ex Fries, identified in Ugandan nuts were shown to produce the toxin *(166)* which was named "Aflatoxin." Subsequently Nesbitt et al. *(143)* and Hartley et al. *(97)* showed that the single fluorescent "aflatoxin" spot could be resolved into four main components by thin layer chromatography on silica gel using chloroform-methanol as the mobile phase. Two spots fluoresced blue and two fluoresced green under UV light, resulting in the assignment of the trivial names, aflatoxins B_1, B_2, G_1 and G_2.

The molecular formulae for aflatoxin B_1 and aflatoxin G_1 were determined as $C_{17}H_{12}O_6$ and $C_{17}H_{12}O_7$, respectively, from elemental analysis and mass-spectral data *(143)*.

STRUCTURE

The structures of aflatoxins B_1 and G_1 (Fig. 1) were determined by Asao et al *(8)*, but without stereochemical designations, and aflatoxins B_2 and G_2 were shown to be the dihydro derivatives of aflatoxin B_1 and G_1, respectively. Total chemical synthesis of aflatoxin B_1 has been achieved by Büchi et al. *(46)*. X-ray crystallographic investigations *(51,204,205)* established the cis-fusion of the two dihydrofuran rings. The absolute configuration of aflatoxins has been established by Brechbühler et al. *(36)*, as shown in Fig. 2. The basic skeleton of the aflatoxin molecule is a condensed bisfuran/coumarin ring system.

Not all strains of *A. flavus* and *A. parasiticus* produce aflatoxins. Toxigenic *A. parasiticus* strains generally produce aflatoxins B_1, B_2, G_1 and G_2, while toxigenic *A. flavus* strains produce B_1 and B_2 toxins. Aflatoxins M_1 and M_2 are typically mammalian metabolites of aflatoxin B_1 and B_2, respectively, though they may also occur in the fungal cultures. Other aflatoxins isolated from *A. flavus* cultures include aflatoxin B_{2a} and aflatoxin G_{2a} *(69)*. Reviews on the occurrence and structure of the various aflatoxins have been published, *(98,101,183-185)*.

Aflatoxin B_1 is the most commonly produced aflatoxin, and is also the most toxic and carcinogenic. Consequently, it has received the most attention.

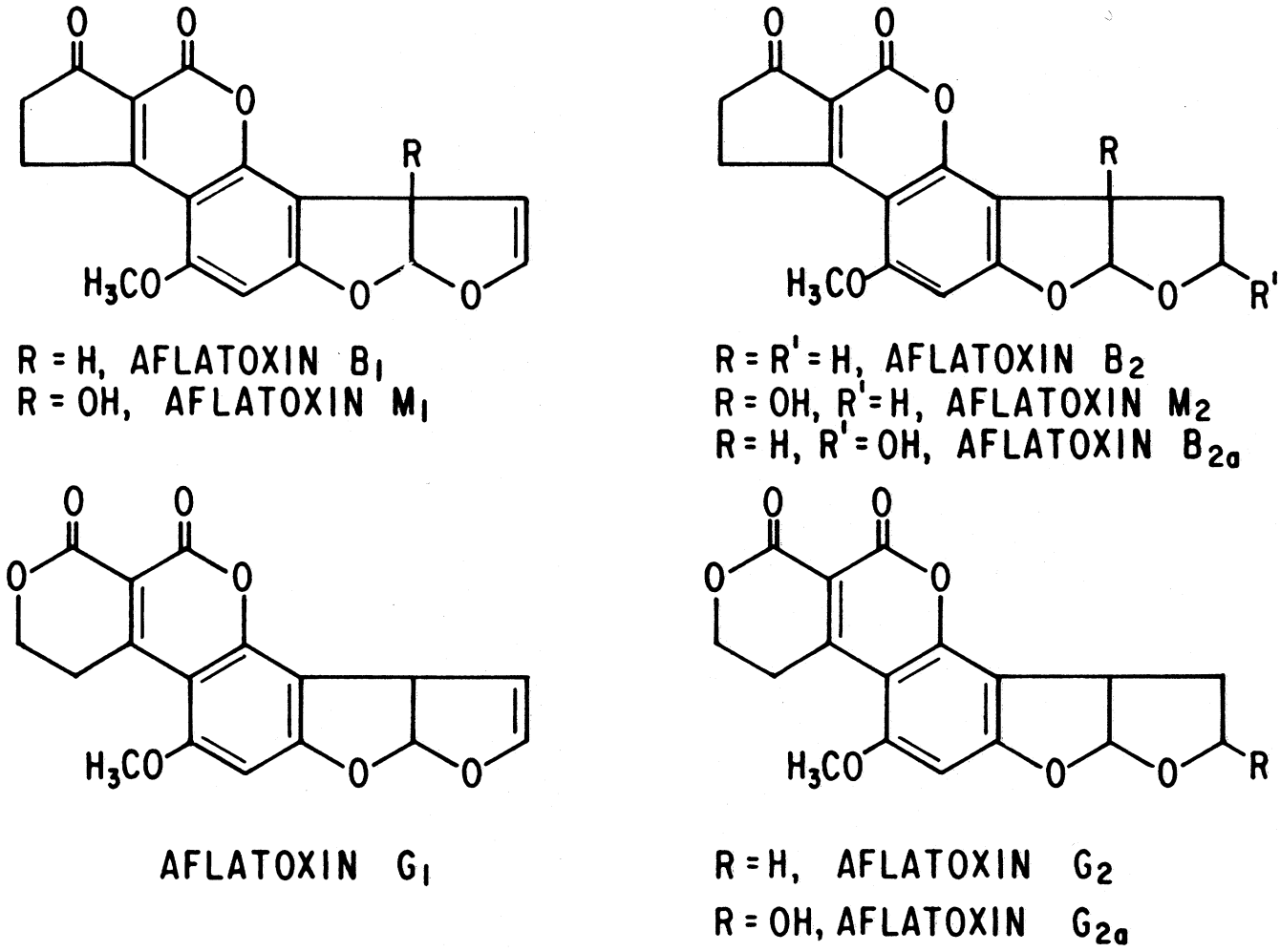


Figure 1. *Structure of the major aflatoxins*

Since the discovery of aflatoxins numerous substances and environmental conditions have been examined in an effort to find an agent that would effectively control growth and aflatoxin production by *A. flavus* and *A. parasiticus*. Accordingly, considerable information is available on production of aflatoxins, both under laboratory conditions and in different food and feed commodities. However, comparatively less is known about the regulatory mechanisms controlling aflatoxin biosynthesis. The purpose of this review is to present information about compounds that regulate or directly affect the biosynthesis of aflatoxins.

BIOSYNTHESIS OF AFLATOXIN B_1

Elucidation of the biosynthetic pathway for aflatoxins has involved intensive studies of the structure of various metabolites of *A. flavus* and *A. parasiticus* as possible intermediate compounds, incorporation of isotopically labelled precursors and the use of *A. parasiticus* mutants which do not produce aflatoxin but accumulate various intermediate compounds. A variety of precursors and biosynthetic pathways has been proposed; however, the details of this anabolic pathway are still largely unknown.

The biosynthetic studies will not be discussed since these have been adequately covered in recent reviews *(6,20,21,101,132,153,183,207)*. Comprehensive monographs dealing with aflatoxins have been published *(89,101)*.

Observations based on experiments with isotope-labelled acetate led to the conclusion that aflatoxin B_1 is an acetate-derived compound *(1,15,34,60,61,99,106,107, 176)*. The acetate-malonate or polyketide origin of aflatoxin B_1 was established unambiguously by Biollaz et al. *(33,34)*. Radioactive aflatoxin B_1, prepared by adding [1-^{14}C]-acetate or [2-^{14}C]-acetate to *A. flavus* cultures, was subjected to selective degradation. They found that the carbon skeleton of aflatoxin B_1 was derived entirely from acetate while the methoxy group carbon was derived from methionine. They determined the origin of 12 of the 16 skeletal carbon atoms. Later experiments led to the assignment of all the carbon atoms *(108,150,182)*. Results indicated that 9 carbon atoms were derived from C-1 of acetate and 7 carbon atoms from C-2 of acetate. The activity of the labelled carbon atoms was equal throughout the basic skeleton, suggesting a single polyacetate

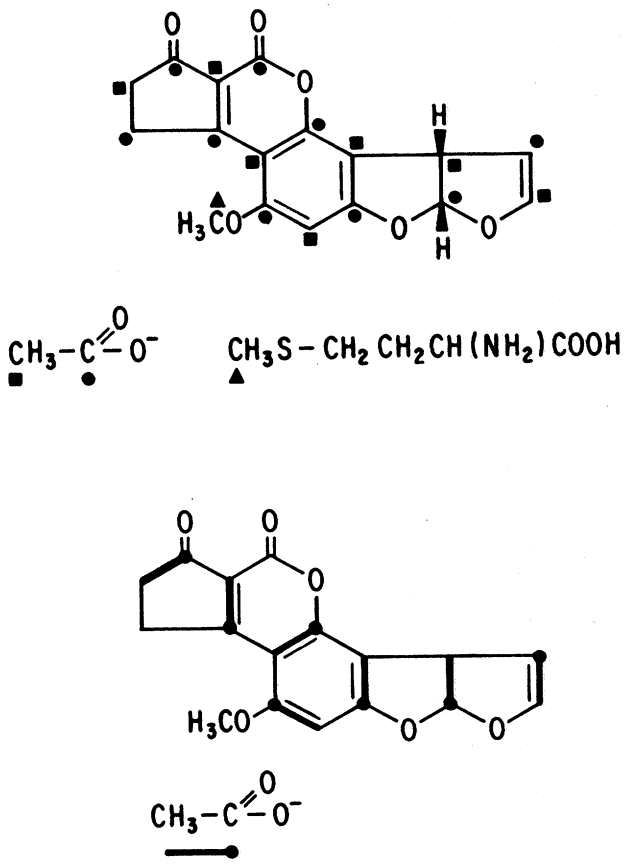


Figure 2. *The origin of the carbon atoms and the arrangement of intacht acetate units in aflatoxin B_1.*

(polyketide) chain. Figure 2 shows the arrangement of intact acetate units in aflatoxin B_1.

At present 6 compounds (Fig. 3) have been recognized as being intermediate compounds in the biosynthesis of aflatoxin B_1-norsolorinic acid, averantin, averufin, versiconal hemiacetal acetate, versicolorin A and sterigmatocystin. A number of these intermediate compounds have been detected in cultures of aspergilli other than *A. flavus* and *A. parasiticus,* particularly *Aspergillus versicolor.* It is of interest to note that many *Aspergillus* species *(58,169)* can produce sterigmatocystin, an intermediate compound thought to occur late in the metabolic pathway (Fig. 3). Other intermediate compounds have been proposed but evidence for their assignment as precursors of aflatoxins has not been firmly established.

Blocked mutant strains of *A. parasiticus* have been developed that accumulate the anthraquinone intermediate compounds norsolorinic acid *(119)*, averantin *(24)*, averufin *(62)* and versicolorin A *(117,118).* Versiconal hemiacetal acetate is produced by the wild-type strain in the presence of the insecticide dichlorvos (*54,82,168, 211*). Several pathways involving polyketide intermediate compounds have been proposed including: (*a*) C_{18} polyketide single unit, (*b*) C_{18} polyketide two units, and (*c*) C_{20} polyketide single unit. Evidence for the various pathways has been reviewed (*6,20,101,153,183*). Experimental evidence presently available indicates that aflatoxin B_1 is synthesized from acetate by way of the C_{20}

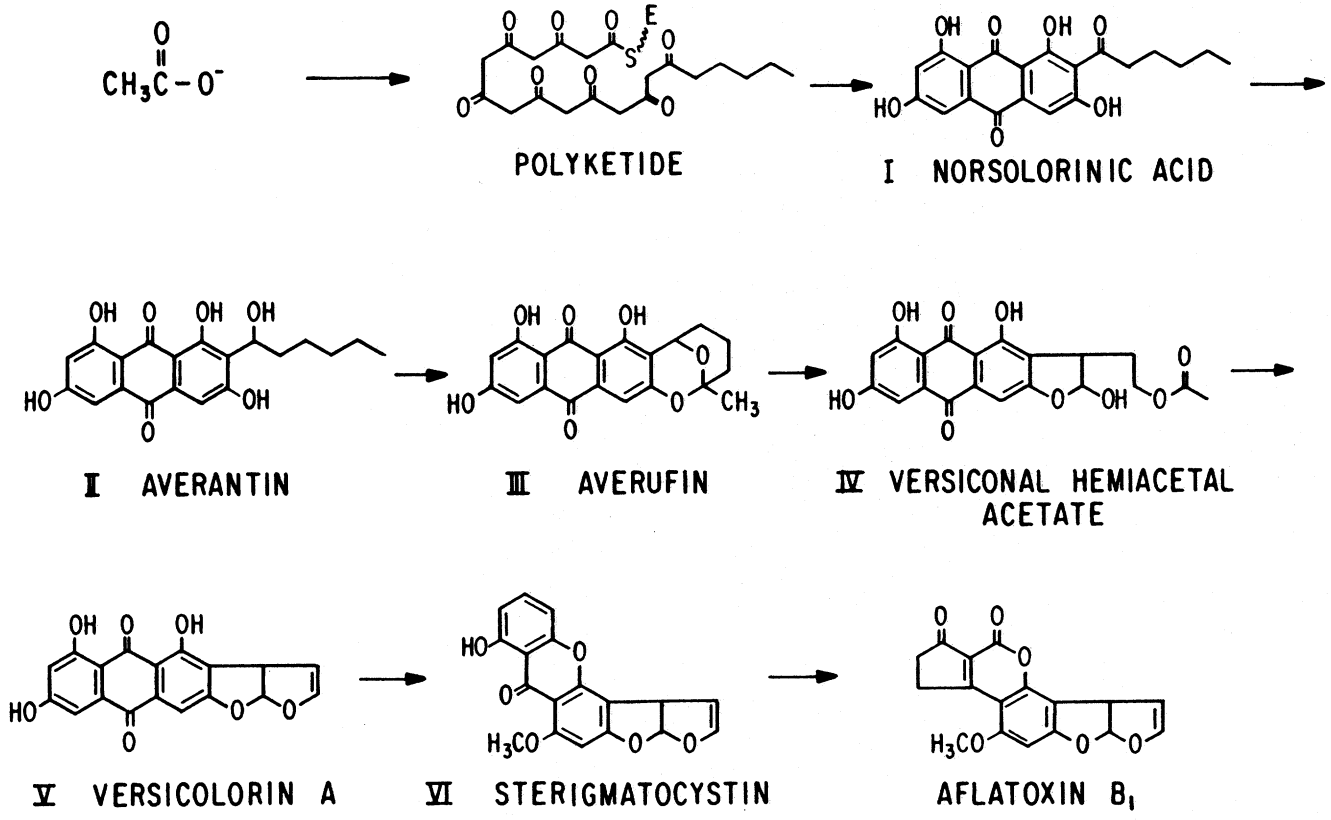


Figure 3. *Pathway for biosynthesis of aflatoxin B_1 and structures of known intermediates.*

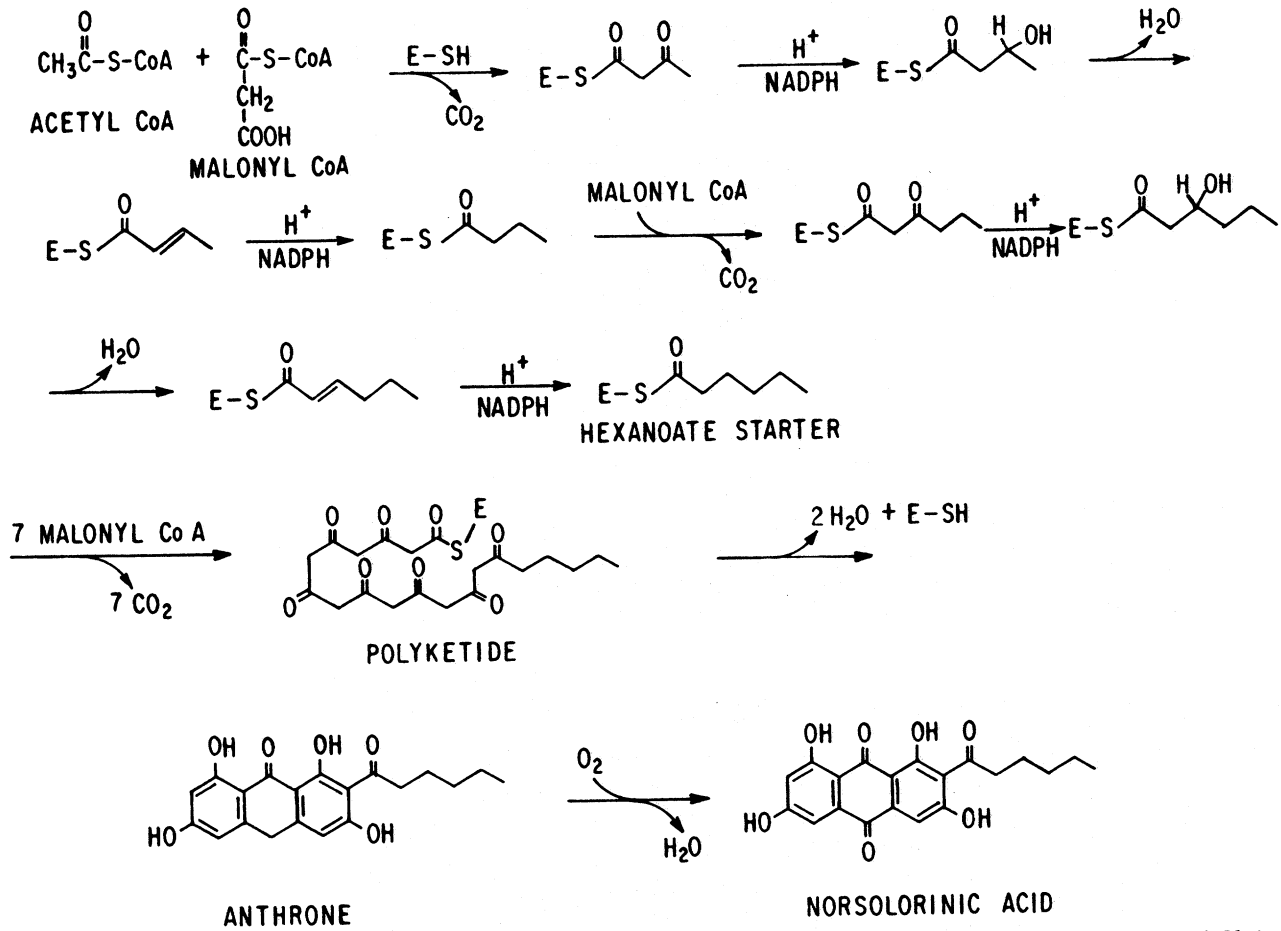


Figure 4. *Scheme for the biosynthesis of norsolorinic acid via a C_{20} polyketide intermediate [adapted from Bennett and Christensen (20)].*

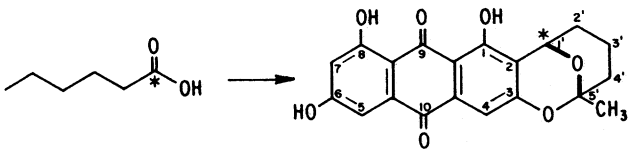


Figure 5. *Incorporation of hexanoic acid into averufin.*

polyketide intermediate compound involving sequential formation of norsolorinic acid, averantin, averufin versiconal hemiacetal acetate, versicolorin A and sterigmatocystin (Fig. 3).

As proposed by Bennett and Christensen *(20)*, the biosynthetic pathway involves one acetyl CoA and 9 malonyl CoA. The first stage of biosynthesis proceeds as in fatty acid biosynthesis with condensation of acetyl CoA and 2 units of malonyl CoA in the presence of NADPH to form a hexanoyl starter. Condensation of this chain with 7 subsequent malonyl CoA units then occurs without intermediate reduction of the polyketide chain. Cyclization and aromatization of the C_{20} polyketide chain results in formation of an anthrone *(206)* which is oxidized to norsolorinic acid (Fig. 4)

Evidence for hexanoate as a starter unit in aflatoxin biosynthesis was recently provided by Townsend and Christensen *(189)* and Townsend et al. *(194)* who obtained intact incorporation of [1-^{13}C] hexanoic acid into averufin, a key C_{20}-anthraquinone intermediate compound (Fig. 5). Townsend et al. *(194)* suggested that an intact hexanoate starter unit may arise from a separate synthetase or from ß-oxidation.

Participation of an anthrone (Fig. 4) in the biosynthetic pathway was indicated by studies examining the incorporation of ^{18}O into averufin by a blocked mutant of *A. parasiticus (206)*. When the mutant was grown in [^{18}0] oxygen gas, the averufin obtained had one ^{18}0 label at C(10) (Fig. 5), but when grown in the presence of [1-^{13}C, $^{18}0_2$] acetate, no ^{18}0 label was found at C(10) while all other oxygens were labelled. These results suggest that the polyketide chain condenses to form the anthrone which then undergoes aerobic oxidation at C(10) to form an anthraquinone derivative, norsolorinic acid.

Experiments had indicated that averufin is incorporated into aflatoxin B_1 by *A. parasiticus*. However, since averufin itself had been biosynthetically derived from labelled acetate, a possibility remained that degradation of the averufin side chain could yield acetate which was subsequently reincorporated to yield aflatoxin B_1 with the same labelling pattern as aflatoxin derived from the respective labelled acetate *(20)*. Townsend et al. *(193)* synthesized specifically labelled samples of averufin and demonstrated their intact incorporation into aflatoxin B_1. The inner and central two acetate units of the averufin

side chain are utilized to construct the bisfuran portion of the aflatoxin B_1 molecule, while the terminal acetate unit is lost. Recent reports *(174,175,190,192,195)* provide additional evidence to support the role of averufin as the key intermediate compound in the aflatoxin biosynthetic pathway.

Townsend et al. *(195)* and Townsend and Christensen *(190)* also showed that nidurufin, a 2′-OH derivative of averufin, is not a precursor of aflatoxin B_1. The absolute configuration of averantin *(191)* and averufin *(116)* has been established and is in accord with the biogenetic pathway for aflatoxin B_1. The total synthesis of (±)-averufin and (±)-nidurufin has been reported *(149)*.

Recent reports indicate that two additional metabolites of *A. parasiticus* and *A. flavus* are probable intermediate compounds in the biosynthetic pathway. Bhatnagar et al. *(27a)* reported that O-methylsterigmatocystin is an intermediate between sterigmatocystin and aflatoxin B_1. McCormick et al. *(138a)* placed averufanin [1,3,6,8-tetrahydroxy-2,2′-(6′-methyltetrahydropyran) anthraquinone] in the biosynthetic pathway between averantin and averufin.

Experimental data indicate that biosynthesis of aflatoxin occurs in the cytoplasm *(106,157,177)*. Conversion of ^{14}C-sterigmatocystin into aflatoxin B_1 was obtained by Singh and Hsieh *(177)* using a cytoplasmic fraction from *A. parasiticus* mycelium. This demonstrated that the enzymes required for at least the latter stages of aflatoxin synthesis are present in the extramitochondrial fraction. The cell-free extract required NADPH for activity, which implied involvement of an oxidation-reduction reaction and suggested that the enzyme involved is an oxygenase *(177)*. Anderson and Dutton *(5)* used cell-free extracts from lysed protoplasts of *A. flavus* to study conversion of ^{14}C-labelled versiconal hemiacetal acetate, versicolorin A and sterigmatocystin into aflatoxin B_1. Conversion of sterigmatocystin was much more efficient than that of versiconal hemiacetal acetate. Conversion of versicolorin A into aflatoxin B_1 could not be demonstrated, presumably due to the absence of an essential cofactor in the cell-free extract. Activity was present in the microsomal fraction isolated from the lysed protoplasts, while the mitochondrial and cell membrane fractions showed no activity. Further evidence for involvement of oxygenases was given.

Studies on biosynthesis of aflatoxins have been mainly focused on the most commonly produced aflatoxin B_1, and biogenesis of aflatoxins B_2, G_1 and G_2 has received comparatively little attention. It has been proposed that aflatoxin B_1 is the precursor of the other commonly occurring aflatoxins *(100,133)*. While many strains that produce only the B-aflatoxins have been isolated from nature, strains that produce only the G-aflatoxins are unknown *(20)*. Therefore most workers concur with Biollaz et al. *(34)* that aflatoxin G_1 is derived oxidatively from aflatoxin B_1. However, strains of *A. flavus* accumulating aflatoxin B_2 but not B_1 have been reported *(91,167)* suggesting that aflatoxin B_1 is not a precursor of aflatoxin B_2. Recent work *(66,68)* showed that aflatoxin B_1 and B_2 originate independently from versicolorin A and versicolorin C, respectively. These workers also showed that aflatoxins M_1 and M_2 arise from aflatoxins B_1 and B_2, respectively.

SUBSTANCES AFFECTING AFLATOXIN PRODUCTION

A number of substances have been identified that inhibit toxin production, but the effect is generally due to inhibition of fungal growth. Table 1 lists studies involving various compounds including antimicrobial agents, antibiotics and pesticides.

Table 2 lists studies involving botanical substances and their components. All of these materials exhibited inhibitory effects at least to some extent.

Table 3 lists compounds that had little or no effect and those that induced increased growth and/or aflatoxin production by *A. flavus* and *A. parasiticus*. The effects of various substances on *A. flavus* and *A. parasiticus* have been reviewed *(57,114,115,161)*.

Results obtained by various investigators are often contradictory. This undoubtedly is at least partially due to differences in the fungal strains, cultural conditions and media employed. For studies involving liquid cultures, aflatoxin content has been reported for the liquid portion of the culture, the mycelium or the total culture. Rarely have results been expressed on the basis of aflatoxin content per unit weight of mycelium obtained. Interpretation of results is also complicated by the varied range of concentrations of test compounds employed. In many instances low concentrations of test compounds have been found to stimulate growth and/or toxin production, while higher concentrations were completely inhibitory *(87, 136,141,201,214)*.

Although most compounds inhibit aflatoxin production by inhibiting fungal growth, a limited number, e.g. dichlorvos, exert little effect on growth but profoundly affect aflatoxin production. These substances are of particular interest in that the study of their effects may aid in elucidation of the mechanisms involved in or regulating aflatoxin biosynthesis. In most instances the research results obtained for various inhibitors were presented only in terms of fungal growth and aflatoxin production. The following is a discussion of test substances (not included in Tables 1-3) that exert some additional effect on the fungus, such as alteration of enzymatic activity, inhibition or stimulation of various metabolic processes or changes in accumulation of metabolites.

Dichlorvos

The insecticide dichlorvos (dimethyl-2,2-dichlorovinyl phosphate) possesses particularly strong anti-aflatoxigenic properties. Other organophosphorus insecticides inhibited aflatoxin production to a lesser extent *(65,105)*. Use of fungicides and pesticides to control aflatoxin production in liquid cultures and in corn have been reviewed by Draughon *(64)*. Detailed studies have been carried out

TABLE 1. *Studies of compounds that inhibit aflatoxin formation largely through inhibition of growth of* A. flavus *and* A. parasiticus.

Substance	Reference	Remarks
p-Aminobenzoic acid	Davis and Diener *(59)*	
Antifungal agents	Holmquist et al. *(104)*	aflatoxin not analyzed
Benzoic acid derivatives	Chipley and Uraih, *(53)*	
t-Butylhydroquinone	Lin and Fung *(125)*	
Butyric acid	Ghosh and Häggblom *(88)*	
Coconut shell smoke	Arseculeratne et al. *(7)*	
Crystal violet	Stewart et al. *(181)*	
Diacetyl	Rama Devi and Polasa *(159)*	total inhib. at 250 ppm
2,6-Dichloro-4-nitroaniline	King et al. *(114)*	MIC[a] 12.5μg/ml
Dimethyl sulfoxide	Bean and Rambo *(18)*	
Fatty acids	Mayura et al. *(138)*	
	Tiwari et al. *(188)*	
Ferulic acid	Sinha and Singh *(179)*	
	Bilgrami et al. *(31)*	cereals and seeds
Fluoroacetic acid	Reiss *(163)*	conidia inhib., no growth at >0.6 mg/ml
Gentian violet	King et al. *(114)*	MIC 10 μg/ml
p-Hydroxylbenzoic acid	Sinha and Singh *(179)*	
Lauric acid derivatives	Rama Devi and Polasa *(158)*	
	Chipley et al. *(52)*	
Malachite green	King et al. *(114)*	MIC 25 μg/ml
Natamycin (pimaricin)	Azzouz and Bullerman *(10)*	
Nystatin	Yousef and Marth *(216)*	
Obtusastyrene (4-cinnamylphenol)	Jurd et al. *(110)*	MIC 100 μg/ml
Pentachlorophenol	King et al. *(114)*	MIC 5 μg/ml
Phenylboric acid	Reiss *(163)*	total inhib. at 1 mg/ml
o-Phenylphenol	Jurd et al. *(110)*	MIC 100 μg/ml
Phloroglucinol	Sinha and Singh *(179)*	
Propionic acid	Ghosh and Häggblom *(88)*	
	Tsai et al. *(196)*	
	Stewart et al. *(181)*	fungicidal at >3.0 μg/ml
	Buchanan and Ayres *(37)*	
	Mallozzi et al. *(134)*	
Propyl paraben	Jurd et al. *(110)*	
Pyrocatechol	Sinha and Singh *(179)*	
Sevin (carbaryl)	Draughon and Ayres *(65)*	
Sodium nitrite	Obioha et al. *(148)*	temporary inhib.; converts NO_2^- to NO_3^-
Sodium chloride	Uraih and Chipley *(201)*	
	Buchanan and Ayres *(37)*	stimulatory at 2 g/100 ml
	El-Gazzar et al. *(72)*	
Sucrose esters	Marshall and Bullerman *(136)*	
6-Thioguanine	Reiss *(163)*	total inhib. at 1000 μg/ml; increased aflatoxin B_1 at 10 μg/ml
Thiram 80	King et al. *(114)*	MIC 5 μg/ml
Tolnaftate	Khan et al. *(113)*	yeastlike form at 100 ppm; total inhib. at 500 ppm
o-Vanillin	Sinha and Singh *(179)*	
	Bilgrami et al. *(32)*	cereals and seeds
Wood smoke	Alverez-Barrea et al. *(4)*	
	Uraih and Ogbadu *(202)*	

[a]MIC = minimum inhibitory concentration.

TABLE 2. *Studies of the effect. of botanical substances and their components on* A. flavus *and* A. parasiticus.

Substance	Reference	Remarks
Anethole	Hitokoto et al. *(102)*	
Caffeic acid	Swaminathan and Koehler *(186)*	
Carrot root extract	Batt et al. *(16)*	raw carrot
Carrot seed oil	Batt et al. *(17)*	
Cinnamaldehyde	Bullerman et al. *(49)*	
Cinnamon	Bullerman *(47)*	
	Hitokoto et al. *(103)*	complete inhibition
	Azzouz and Bullerman *(10)*	complete inhibition
Cinnamon oil	Bullerman et al. *(49)*	
Citral	Batt et al. *(17)*	
Citrus oils	Karapinar *(112)*	
	Alderman and Marth *(2)*	
Clove oil	Bullerman et al. *(49)*	
Corn ear volatile compounds	Gueldner et al. *(90)*	
5,7-Dimethoxyisoflavone	Turner et al. *(198)*	
Eugenol	Bullerman et al. *(49)*	
	Hitokoto et al. *(102)*	
Geraniol	Batt et al. *(17)*	
β-Ionone	Wilson et al. *(209)*	
	Gueldner et al. *(90)*	
Limonene	Batt et al. *(17)*	
	Alderman and Marth *(2)*	
Marine algae (volatile oils)	Mabrouk et al. *(130)*	
o-Methoxycinnamaldehye	Morozumi *(140)*	complete inhibition at 100 μg/ml
Onion extracts	Sharma et al. *(172)*	
Pepper *(Piper nigrum)*	Madhyastha and Bhat *(131)*	as substrate
	Seenappa and Kempton *(170)*	as substrate
Pepper oil	Madhyastha and Bhat *(131)*	
Phytoalexins	Wotton and Strange *(210)*	inhibit spore germination
Piperine	Madhyastha and Bhat *(131)*	
Plant extracts	Bahk and Marth *(13)*	honeysuckle flower inhibitory
	Salomao and Purchio *(165)*	sisal inhibitory
	Hitokoto et al. *(103)*	
	Bilgrami et al. *(29)*	
	Bilgrami et al. *(30)*	*Ricinus communis* complete inhib.
Saponins	Bahk et al. *(14)*	
Saponin (ginseng)	Bahk and Marth *(12)*	
Terpinene	Batt et al. *(17)*	
Terpineol	Batt et al. *(17)*	
Thiopropanal-S-oxide	Sharma et al. *(172)*	lachrymator from onion
Spices	Azzouz and Bullerman *(10)*	
	Hitokoto et al. *(103)*	
	Hitokoto et al. *(102)*	
	Flanigan and Hui *(83)*	as substrates
	Karapinar *(112)*	
	Llewellyn et al. *(126)*	as substrates
	Llewellyn et al. *(127)*	as substrates
	Mabrouk and El-Shayeb *(129)*	
Thymol	Hitokoto et al. *(102)*	
	Buchanan and Shepherd *(40)*	
White potato	Swaminathan and Koehler *(186)*	a phenolic acid

TABLE 3. *Studies of compounds that have no effect or that stimulate growth and/or aflatoxin formation by* A. flavus *and* A. parasiticus.

Substance	Reference	Remarks
Acetone	Bennett et al. *(23)*	resting cells; aflatoxin stimulated at <1M
	Floyd et al. *(84)*	stimulatory at 0.1%
BHT (butylated hydroxytoluene)	Foudin et al. *(85)*	no effect
	Fung et al. *(86)*	no effect
	Lin and Fung *(125)*	no effect
Ethanol	Bennett et al. *(23)*	resting cells; aflatoxin stimulated at <1M
DL-Ethionine	Reiss *(163)*	no effect on growth at 1000 μg/ml; enhanced growth at 10 μg/ml
Isoprothiolane	Yousef and Marth *(216)*	Stimulates aflatoxin B_1 accumulation
Methoxychlor	Draughon and Ayres *(65)*	100 mg/L inhibits aflatoxin by 6%
Nisin	Yousef et al. *(213)*	initial delay of growth followed by stimulation of toxin
Phytate	Ehrlich and Ciegler *(70)*	no effect at pH 4.5; aflatoxin stimulated at pH 6.0
	Ehrlich and Ciegler *(71)*	no effect; degradation to phosphate
Propyl gallate	Lin and Fung *(125)*	no effect
Pyridazinone herbicides	Bean and Southall *(19)*	no effect on growth (20-60 μl/ml)
Steroidal carbamates	Jung and Suh *(109)*	no effect on growth; aflatoxin inhib.

with dichlorvos, and this compound was instrumental in helping to establish the biosynthetic pathway of aflatoxin B_1.

Rao and Harein *(160)* found that aflatoxin production on rice, corn, wheat and peanuts was inhibited in the presence of 20 ppm of dichlorvos. Hsieh *(105)* reported that biosynthesis of aflatoxin by *A. parasiticus* was strongly inhibited by dichlorvos in liquid media. A concentration of 10 ppm of dichlorvos inhibited 90% of aflatoxin production without affecting fungal growth. When actively synthesizing cultures of *A. parasiticus* were supplemented with 10 ppm of dichlorvos and [1-^{14}C]acetate, a reduced amount of aflatoxin B_1 was produced which contained almost no label from the acetate. This suggested that dichlorvos inhibits an early step in the pathway leading to biosynthesis of aflatoxin *(105)*.

Reduction in the yield of aflatoxin in the presence of dichlorvos was accompanied by formation of an orange pigment, tentatively identified by Schroeder et al. *(168)* as "versiconal acetate." The orange pigment was converted into aflatoxin B_1 by untreated mycelium of *A. parasiticus (211)*. Also, in the presence of dichlorvos, sterigmatocystin was converted into aflatoxin B_1, but averufin was converted into the orange pigment instead of into aflatoxin B_1. This indicated that dichlorvos blocks an enzymatic step in the pathway of aflatoxin B_1 biosynthesis that lies beyond averufin but before sterigmatocystin *(211)*. Schroeder et al. *(168)* suggested that dichlorvos inhibits biosynthesis of the bisfuran ring system.

Using various precursors of aflatoxin B_1 and blocked mutants of *A. parasiticus,* Singh and Hsieh *(178)* showed that dichlorvos inhibited conversion of "versiconal acetate" to versicolorin A. They proposed a pathway for the biosynthesis of aflatoxin B_1 to be the sequence: acetate → averufin → versiconal acetate → versicolorin A → sterigmatocystin → aflatoxin B_1.

Bennett et al. *(22)* reported that in the presence of 10-100 ppm dichlorvos production of versicolorin A was inhibited in a mutant strain of *A. parasiticus* that does not produce aflatoxin but accumulates versicolorins. Production of aflatoxin and versicolorin A in a wild-type strain was inhibited under the same conditions. Addition of dichlorvos caused only moderate inhibition of averufin and versicolorin C production. Additions of ^{14}C-versicolorin A to resting cells of the wild-type strain of *A. parasiticus* resulted in 34.5% incorporation of label into aflatoxin B_1 which was lowered to 13.0% in the presence of 20 ppm dichlorvos, indicating only partial blockage of this reaction.

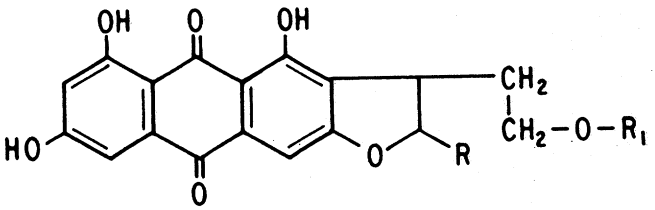
Fitzell et al. *(82)* synthesized the orange pigment, described by Schroeder et al. *(168)* as "versiconal acetate," from ^{13}C-labelled acetate using dichlorvos-treated cultures

of *A. parasiticus*. With the aid of nuclear magnetic resonance studies they established the structure of this pigment, solely derived from acetate, as versiconal hemiacetal acetate and demonstrated its biosynthetic relationship to aflatoxin B_1.

Dutton and Anderson *(67)* determined that addition of dichlorvos to shake cultures of *A. flavus* resulted in inhibition of aflatoxion production and in formation of a group of seven related anthraquinone pigments. Three of these were produced in relatively large quantities: versiconal hemiacetal acetate, versicolorin C and a previously unknown pigment named versicol for which the structure I shown in Fig. 6 was suggested on the basis of mass-spectral data. The other four anthraquinones were produced in smaller quantities: averufin, averufanin, versiconol and a previously unknown compound tentatively identified as versicol acetate (structure II, Fig. 6). These investigators tested the effect of variety of organophosphorus compounds on growth and aflatoxin production of *A. flavus*. Five of the compounds (Chlormephos, Ciodrin, Naled, Phosdrin and Trichlorphon) at concentrations of 20 and 100 μg/ml of culture exhibited activity similar to that of dichorvos. Dutton and Anderson *(67)* examined the structure of the test compounds and postulated that a phosphate ester group and an electron deficient carbon atom one removed from the phosphorus atom are necessary components for a compound to possess inhibitory activity against aflatoxin biosynthesis. They pointed out that dichlorvos inhibited esterase and oxygenase activities in cell-free extracts from *A. flavus* and suggested that a particularly sensitive esterase or oxygenase may be the cause of the specific metabolic block in aflatoxin biosynthesis.

Tridecanones

Tridecanones were reported to exhibit dichlorvos-type activity *(67)*. Addition of 2- or 3-tridecanone to cultures of *A. flavus* resulted in accumulation of anthraquinone pigments and a decrease in aflatoxin. The authors suggested that these compounds, which are insoluble in water but are metabolized by the mold, may act by solvating the anthraquinones. This would allow the anthraquinones to pass through the cell membrane, thereby preventing them from acting as intermediate compounds in the aflatoxin biosynthetic pathway. Dutton and Anderson *(67)* suggested further that tridecanones may compete for oxygenases present in the hydrophobic regions of the cell and hence by saturation effect limit the rate of anthraquinone metabolism.



I R = R_1 = H

II R = H, R_1 = $COCH_3$

Figure 6. *Anthraquinone pigments produced by* A. flavus *in cultures containing dichlorvos* (67).

Selenite

Badii et al. *(11)* made an interesting observation that sodium selenite (40 μg/ml) induced formation of an orange pigment by mycelia of a variety of *Aspergillus* species grown on Czapek Dox agar. Growth was also inhibited to various degrees. In liquid medium aflatoxin production by *A. parasiticus* increased slightly in the presence of low concentrations of sodium selenite but was inhibited by concentrations > 200 mg/L. The intensity of pigmentation increased with increasing concentration of selenite and was inversely proportional to mycelial weight. The pigmentation decreased with increasing incubation time once the fungus established normal growth, which was delayed due to inhibition of spore germination. Selenite was rapidly taken up by the mycelium. The identity of the orange pigment was not established but its transient nature suggests that it might be involved in aflatoxin biosynthesis.

Nitrate

Kachholz and Demain *(111)* studied the control mechanisms that regulate formation of aflatoxin using a blocked mutant of *A. parasiticus* that accumulates averufin, an early intermediate compound of aflatoxin biosynthesis. They found that biosynthesis of averufin was regulated by the nitrogen source used for growth. Nitrate exerted a negative effect, while ammonium ion favored averufin formation. The suppressive effect of nitrate on averufin formation was not due to pH changes nor to increased energy requirement to mediate reduction of nitrate to ammonium. Instead, the effect was attributed to a repression of enzyme(s) involved in averufin formation. Growth in the presence of nitrate resulted in repression of averufin synthesis; however, when $NaNO_3$, $(NH_4)_2HPO_4$ or no nitrogen source was added to resting cells treated with cycloheximide, similar levels of averufin were obtained, indicating that nitrate does not inhibit preformed enzymes of averufin biosynthesis. Wild-type *A. parasiticus* grown in the presence of NO_3^- produced much less aflatoxin than similar cultures containing NH_4^+. This indicated that inhibition of aflatoxin biosynthesis is due to the regulatory effect of nitrate on averufin formation. Bennett et al. *(25)* found that versicolorin A and C production by a blocked mutant of *A. parasiticus* was completely inhibited by use of $NaNO_3$ as a nitrogen source. However, $(NH_4)_2SO_4$ and NH_4NO_3 supported greater growth and versicolorin production.

Dutton and Anderson *(67)* reported that $NaNO_3$ (10-15%) elicited dichlorvos-type activity when added to 2-d-old cultures of *A. flavus*. Anthraquinone pigments were obtained, while aflatoxin levels were decreased compared with control cultures.

Ethylene

Sharma et at. *(171)* suggested that ethylene, which is produced by *A. flavus* and *A. parasiticus* during early phases of growth, may be involved in regulation of aflatoxin biosynthesis. The onset of toxin formation was marked by the absence of ethylene evolution. Addition of 2-chloroethylphosphonic acid (CEPA), an ethylene-generating compound, inhibited aflatoxin biosynthesis in vivo. Low concentrations of CEPA slightly stimulated growth while high concentrations were inhibitory. The authors noted that no known precursor of aflatoxin accumulated in the presence of CEPA, suggesting that the action of the compound may involve an early stage of polyketide synthesis.

Benzoic acid

Uraih et al. *(200)* reported that aflatoxin production by *A. flavus* was greatly reduced by benzoic acid or sodium benzoate in synthetic media. The reduction was accompanied by accumulation of a yellow pigment which was characterized as closely related to an acetyl derivative of a versiconal-type compound. The yellow pigment was converted to aflatoxin B_1 by a cell-free extract prepared from *A. flavus* grown in synthetic media. However, when benzoic acid or sodium benzoate (8 mg/ml) was added or when the cell-free extract was autoclaved, conversion of the yellow pigment to aflatoxin B_1 was prevented. These results indicated that the yellow pigment may be an intermediate compound in the biosynthesis of aflatoxin B_1 and that benzoic acid blocks an enzymatic step in the biosynthetic pathway. This pigment has not been characterized further. In studies on the effect of other acids and their salts Uraih and Chipley *(201)* noted that a reduction of aflatoxin formation was generally accompanied by accumulation of an unidentified orange pigment, while stimulation of aflatoxin production was accompanied by unidentified blue and green flourescing compounds which had R_f values lower than aflatoxins B_1, G_1, B_2 and G_2. In a recent study, Valcarcel et al. *(203)* were unable to verify the former findings that *A. parasiticus*, treated with benzoate, accumulates a yellow pigment of relevance to aflatoxin biosynthesis.

Oxygen

Increased aeration of *Aspergillus* cultures resulted in decreased aflatoxin production *(173)*. Aspergilli can catabolize glucose either by way of the Embden-Meyerhof or the hexose monophosphate pathways and can use these systems simultaneously. Aerobic conditions favor use of the hexose monophosphate pathway, while increasingly anaerobic conditions favor the Embden-Meyerhof pathway. Shih and Marth *(173)* concluded that the amount of aflatoxin synthesis depends on the pathway by which glucose is catabolized by the mold. They showed that less toxin was formed in extensively aerated cultures of *A. parasiticus* and the efficiency of ^{14}C-incorporation from [1-^{14}C]glucose was less than that observed in stationary cultures. They proposed that in less aerobic environments oxidation of acetate (via the citric acid cycle) would be decreased and more acetate, which is a precursor of aflatoxin, would be available for synthesis of more aflatoxin *(173)*. Further, as the environment becomes less aerobic, formation of NADPH via the hexose monophosphate pathway would be decreased. Niehaus and Dilts *(145)* have proposed that a low NADPH/NADP ratio favors aflatoxin synthesis.

Azide

Shih and Marth *(173)* also showed that addition of NaN_3, an inhibitor of terminal electron transfer, caused an increase in both aflatoxin and lipid synthesis by *A. parasiticus* due to inhibition of oxidative respiration. They concluded that accumulation of acetate and NADPH favored aflatoxin and lipid synthesis, though the specific role of NADPH levels as a bioregulator of aflatoxin synthesis is unclear *(145)*.

Epoxy derivatives

Compounds containing an epoxy group have been reported to stimulate aflatoxin production. Cerulenin (2,3-epoxy-4-oxo-7,10-dodecadienamide) and tetrahydrocerulenin (2,3-epoxy-4-oxo-dodecamide) significantly stimulated aflatoxin production by *A. parasiticus* when added (100 μg/ml) during the log growth phase *(78)*. Further growth was not inhibited and no difference was found in the content of lipid fractions of the mycelia or of their fatty acid composition. The dihydro and hexahydro derivatives, which do not contain an epoxy group, had no effect *(78)*.

Nomura et al. *(147)* found that cerulenin, an antibiotic isolated from the culture filtrate of the fungus *Cephalosporium caerulens*, markedly inhibited incorporation of ^{14}C-acetate into sterols and fatty acids by the yeast *Candida stellatoidea*, but had no significant effect on incorporation of ^{32}P-phosphoric acid into nucleic acids, ^{14}C-amino acids into proteins, or ^{14}C-glucosamine and ^{14}C-mannose into the cell wall, and did not prevent exogenous respiration of the yeast. These workers suggested that cerulenin affected lipid metabolism, especially biosynthesis of steroids and fatty acid. D'Agnolo et al. *(55)* determined that cerulenin inhibits the fatty acid synthetase of *Escherichia coli* by specifically inhibiting β-ketoacyl-acyl carrier protein synthetase, the enzyme which catalyzes the condensation reaction of fatty acid biosynthesis. Tetrahydrocerulenin also exhibited the same effect; however, the dihydro and hexahydro-derivatives lacking the epoxy group did not inhibit these enzyme systems.

In contrast to the stimulatory effect observed by Fanelli et al. *(78)*, Chipley et al. *(52)* reported that cerulenin (8 μg/ml) was the most effective inhibitor of a number of fatty acid derivatives examined, reducing mycelial growth of *A. flavus* and *A. parasiticus* by 37 and 49%, respectively, and completely inhibiting extracellular accumulation of aflatoxin. The difference in results obtained by the two groups of workers may be due to differences in

fungal strain, media and times of addition of the test compound.

Fanelli et al. *(79)* also showed that epoxy fatty acid derivatives, methyl 9,10-epoxystearate and methyl 9,10:12,13-diepoxystearate, strongly stimulated aflatoxin production by *A. flavus* and *A. parasiticus* when added to culture media either with the inoculum or at the end of the exponential growth phase, while the unsaturated parent compounds or hydroxy derivatives resulting from epoxide ring opening had no effect. No significant differences were found in the content of lipid fractions or in their fatty acid composition of the mycelia grown in the presence of any of the fatty acid derivatives.

According to Fabbri et al. *(74)*, T-2 toxin, a trichothecene mycotoxin produced by various *Fusarium* spp. and characterized by the 12,13-epoxy-trichothec-9-ene ring system, stimulated aflatoxin production by *A. parasiticus* while having little effect on growth. Aflatoxin production increased with increasing concentration of T-2 toxin (0.05 to 15 mg/50 ml) in the medium. The authors pointed out that direct involvement of the epoxide group in aflatoxin biosynthesis is speculative since persistence of T-2 toxin in the medium was not monitored and the possibility that a breakdown product of T-2 toxin may be responsible for the observed effect could not be ruled out.

The mechanism by which epoxy derivatives stimulate aflatoxin synthesis is not known, but one possibility is that they are stimulating cytochrome P-450 type reactions. Bhatnagar et al. *(28)* reported that phenobarbitone strongly stimulated aflatoxin production and hypothesized that this was due to an induced increase in cytochrome P-450 activity. In mammalian systems, epoxy compounds are well known for their ability to induce increased cytochrome P-450 activity.

Peroxy derivatives

The role of lipids in aflatoxin production has been investigated. Supplementation of liquid culture media of *A. flavus* with synthetic triglycerides, fatty acids and sterols showed that these lipid fractions acted as carbon sources *(80)* promoting growth of the fungus without enhancing aflatoxin production *(81)*. However, aflatoxin production by *A. flavus* and *A. parasiticus* on oil seeds was much higher than on starchy seeds *(73)*. The content of polyunsaturated fatty acids in the oils was judged to be an important determinant of aflatoxin production because these fatty acids are more easily peroxidizable than monounsaturated acids. Thus, aflatoxin production by *A. parasiticus* on sunflower seeds (44% oil, containing 75.5% linoleic acid) was 712 μg/g of seed while on peanut (48% oil, containing 29% linoleic acid) it was 335 μg/g of seed after 30 d of incubation *(73)*.

The idea that oxidation of unsaturated lipid plays an important role in induction of aflatoxin production by *A. flavus* and *A. parasiticus* was further supported by experiments using aged seeds *(152)*. The amount of aflatoxin produced was directly related to the peroxide number of the oil content of the seeds. *A. parasiticus* produced 540 μg/g on sunflower seeds aged 1 year (Peroxide # = 0.8) and 3206 μg/g on seeds aged 3 years (Peroxide # = 17.0) after 30 d of incubation. During oxidation of unsaturated lipids epoxides may form to a limited extent, but the main products are lipoperoxides which can undergo further degradation. Addition of 0.1 to 0.5 mg of linoleic acid hydroperoxides (a mixture of ca. 30% 9-hydroperoxyoctadeca-10,12-dienoic acid and ca. 70% 13-hydroperoxyoctadeca-9,11-dienoic acid)/ml to synthetic media increased aflatoxin formation by *A. parasiticus* and *A. flavus* without significantly affecting fungal growth or lipid content of the mycelium. Linoleic acid was employed as a control compound and had no significant effect on aflatoxin production *(73,152)*. A 250-fold increase in aflatoxin production was found in the presence of 0.5 mg of linoleic acid hydroperoxides/ml after 12 d of incubation *(152)*.

Halomethanes

It is also possible to enhance production of aflatoxin by use of substances capable of inducing lipoperoxidation of internal membranes of the fungi. Passi et al. *(151,152)* and Fanelli et al. *(77)* found that halomethanes greatly stimulated aflatoxin production by *A. parasiticus* and *A. flavus*. According to Fanelli et al. *(77)*, the stimulatory effect, $BrCCl_3 > CCl_4 > HCCl_3$, can be correlated with the ease of homolytric bond dissociation ($\chi CCl_3 \rightarrow \chi^{\bullet} + CCl_3^{\bullet}$). Dihalomethanes were much less stimulatory *(151)*.

It has been postulated *(162)* that the basis of toxicity of the hepatotoxin CCl_4 is peroxidation of lipids of the endoplasmic reticulum of liver cells, and that this lipoperoxidation must be induced by the highly reactive trichloromethyl radical ($CCl_3^{\bullet}$) formed by the interaction of CCl_4 with the NADPH-cytochrome P-450 system. The presence of a NADPH-cytochrome P-450 reductase has been established in the microsome of *A. parasiticus (28)*. Support for this hypothesis in *Aspergillus* was found by results obtained on addition to the halomethane-containing cultures of drugs known to act on the cytochrome P-450 system. Thus, phenobarbital enhanced the stimulatory effect of halomethanes *(151,152)*. Fanelli et al. *(77)* studied the effect of halomethanes on the peroxidase activity of *A. parasiticus*. They found that during halomethane-enhanced aflatoxin production peroxidase activity was inhibited, with total inhibition of enzyme activity being observed after 10 d of incubation. Doyle and Marth *(63)* hypothesized that this enyme may be involved in the degradation of aflatoxin, the rate of aflatoxin degradation being greater with greater peroxidase activity.

Rubratoxin B

Rubratoxin B, a metabolite of *Penicillium rubrum* Stoll caused almost complete inhibition of growth of *A. parasiticus* at a concentration of 100 μg/ml in a synthetic medium, while at lower concentrations it was slightly stimulatory to aflatoxin production *(141)*. Involvement of rubratoxin B in secondary metabolism is suggested by the

observation that *Aspergillus niger* accumulates large amounts of pigments in the presence of rubratoxin B concentrations slightly inhibitory to growth *(141)*.

Sorbic acid

The common food preservative sorbic acid (trans, trans-2,4-hexadienoic acid) and its salts inhibit growth and aflatoxin production of *A. flavus* and *A. parasiticus* *(27,48,52,87,154,155,196,214)*. However, subinhibitory levels of sorbate have been reported to stimulate aflatoxin production. Accumulation of aflatoxin B_1 and G_1 by *A. parasiticus* in media containing 100 or 200 ppm potassium sorbate was greater than in control media *(214)*. Growth of *A. parasiticus* was completely inhibited by 0.1% sorbate at pH 4.0 in a synthetic medium; however, at pH 6.0 growth occurred with levels of aflatoxins higher than those in control media *(136)*. Gareis et al. *(87)* reported that although growth of *A. flavus* was prevented and delayed by 0.1 and 0.05% concentrations of sorbic acid, respectively, aflatoxin production in the presence of subinhibitory concentration of sorbic acid (0.025%) was higher than in control media. Pupovac-Velikonja et al. *(155)* noted an increase in growth of *A. parasiticus* in apple juice in the presence of 0.01% potassium sorbate, but a decrease in aflatoxin production. Aflatoxin B_1 was not detected and levels of aflatoxin G_1 were 3 to 5 times smaller than in control medium, which contained more aflatoxin G_1 than B_1. Chipley et al. *(52)* concluded that inhibition by sorbic acid was nonspecific, affecting both mycelial growth and extracellular aflatoxin accumulation to approximately the same extent.

The mechanism by which sorbate inhibits microorganisms has not been satisfactorily elucidated at the present time, and information on its effects on *A. flavus* and *A. parasiticus* is practically nonexistent. Przybylski and Bullerman *(154)* observed that exposure of *A. parasiticus* conidia to inhibitory concentrations of sorbic acid resulted in loss of viability and depletion of ATP. Sofos and Busta *(180)* reviewed the various studies related to the mechanism of inhibition of microorganisms by sorbate and considered the antimicrobial activity of sorbate in light of some proposed mechanisms of action of fatty acids in general. Recently Liewen and Marth *(124)* reviewed the factors that influence the antimicrobial effectiveness of sorbate in foods.

Yousef and Marth *(215)* studied the effect of various antifungal agents on incorporation of [^{14}C]-acetate into aflatoxin by resting cultures of *A. parasiticus*. Incorporation of label was inhibited in the presence of 200 ppm sorbic acid, and the inhibition was greater for aflatoxin B_1 than for G_1. Yousef and Marth *(215)* suggested that sorbic acid inhibited aflatoxin biosynthesis by inhibiting transfer of substances from the growth medium into the cell. Gareis et al. *(87)* proposed that the stimulatory effect of subinhibitory levels of sorbic acid may be due to inhibition of activity of the tricarboxylic acid cycle which in turn leads to an increase in acetylcoenzyme A concentration which is required for initiation of the aflatoxin biosynthetic pathway. However, these authors did not present experimental evidence for their proposal. Inhibition of enzymes such as fumarase, aspartase and succinic dehydrogenase by sorbate has been reported *(212)*. On the other hand, Harada et al. *(96)*, studying the sorbate-induced inhibition of respiration in yeast, suggested that sorbate competes with acetate at the site of formation of acetylcoenzyme A.

BHA

The antioxidant butylated hydroxyanisole (BHA) inhibited growth and aflatoxin production by *A. parasiticus* and *A. flavus* *(50,85,86,125)*. Production of the G aflatoxins was affected to a greater extent than the B aflatoxins *(86,125)*. A possible explanation of this differential response is that BHA inhibits the oxidative process that has been proposed to be responsible for conversion of B aflatoxins to G aflatoxins. Yousef and Marth *(215)* studied the effect of BHA (30 ppm) on the incorporation of ^{14}C-acetate into aflatoxin by resting cultures of *A. parasiticus*. Incorporation of label into aflatoxin B_1 was inhibited by 56.9% and into aflatoxin G_1 by 91.6%. These authors suggested the possibility of involvement of an oxygenase *(5,177)* as the site of inhibition of BHA. The inhibitory effect of BHA may also be due to leakage of intracellular components, as has been suggested for *Pseudomonas (56)*.

Ethoxyquin

The antioxidant ethoxyquin (6-ethoxy-1,2-dihydro-2,2,4-trimethylquinoline), used as a preservative in feed mixes, had little if any effect on growth of seven strains of *A. flavus* and *A. parasiticus* in liquid medium at concentrations of up to 150 ppm *(85)*. However, results obtained by these workers suggest that ethoxyquin acts as a metabolic inhibitor in conversion of aflatoxin B_1 to G_1 in isolates of *A. parasiticus* producing both aflatoxins B and aflatoxins G. Similar findings were reported by Ruprich and Piscac *(164)* for an *A. flavus* strain producing aflatoxins B_1, G_1 and M_1, on a cereal medium. On the other hand, Floyd et al. *(84)* were unable to confirm reports that ethoxyquin inhibited formation of G aflatoxins possibly because they used acetone as a solvent for ethoxyquin. Acetone has been reported to stimulate aflatoxin production *(23,84)*.

Potassium sulfite

Davis and Diener *(59)* found that addition of up to 4% K_2SO_3 to yeast extract-sucrose medium did not inhibit growth of *A. parasiticus*. However, all concentrations of potassium sulfite tested inhibited aflatoxin production to some extent, with levels of $\geq$ 2% yielding virtually complete inhibition. Sulfite is known to inhibit a number of metabolic pathways in fungi, including that of ethyl alcohol production by yeast, where it acts as an aldehyde trapping agent.

Potassium fluoride

Potassium fluoride added to yeast extract-sucrose

medium inhibited growth and aflatoxin production by *A. parasiticus (59)*. At a level of 4% KF, aflatoxins were not detected, and growth was reduced by 60%. Fluoride inhibits glycolytic enolase activity as well as enzymes of several other metabolic pathways in fungi. It also inhibits most phosphatases. Davis and Diener *(59)* concluded that this supports the proposed involvement of glycolysis in aflatoxin formation.

Trace metals

The role of trace metals in biosynthesis of aflatoxins has been studied extensively but is still not well understood. Conflicting results have been reported as to the effect of a given metal on growth and aflatoxin production by *A. flavus* and *A. parasiticus*. Maggon et al. *(132)* reviewed the role of trace metals in aflatoxin biosynthesis. Recent studies were reported by Tiwari et al. *(187)*, Rabie et al. *(156)* and Bennett et al. *(25)*. Apparently magnesium, manganese, molybdenum, iron and zinc are important and are generally included in defined growth media for the aspergilli *(1)*. Increased incorporation of [1-^{14}C]-acetate into aflatoxin in the presence of Zn^{2+}, Mg^{2+}, Mn^{2+}, Ba^{2+}, and Ca^{2+} by resting cells of *A. parasiticus* was observed by Gupta et al. *(94)*. The effect of a large number of inorganic salts on incorporation of [1-^{14}C]-acetate into aflatoxin by spheroplasts and spheroplast lysates from *A. parasiticus* has been reported *(199)*.

Zinc is an essential element for cellular growth and metabolism *(75)* and is essential for aflatoxin biosynthesis *(93,120,135,137)*. Mateles and Adye *(137)* determined that zinc is required at levels of at least 0.4 mg/L for aflatoxin production by *A. flavus,* while Lee et al. *(120)* reported optimum fungal growth and maximum aflatoxin production on a basal medium containing 0.8 mg of zinc/L. Requirements for levels of zinc as high as 50 mg/L have been reported *(132)*. The following experimental evidence indicates that zinc may exert its effect on biosynthesis of aflatoxin by regulating formation of intermediate compounds in the biosynthetic pathway. Using an aflatoxin-blocked mutant strain of *A. parasiticus* that accumulated versicolorin A and versicolorin C, Bennett et al. *(25)* showed that zinc was essential for versicolorin production. Niehaus and Failla *(146)* reported that production of versicolorin by a mutant strain of *A. parasiticus* required zinc concentrations in excess of those required for growth. Maximal grwoth occurred with 2 μM of zinc while maximal versicolorin production required 5 μM of zinc in the medium. Failla and Niehaus *(76)* investigated the relationship between Zn^{2+} uptake, stimulation of versicolorin A production and the time at which Zn^{2+} is added to the fungal culture. Stimulation of versicolorin A production was found only if the supplemental zinc was present during the period of early vegetative growth, 20-30 h post inoculation. Versicolorin synthesis, which began about 50 h post inoculation was directly proportional to the zinc content of mycelia at 30 h. These results suggested that zinc may be acting at the pre-transcriptional or transcriptional level.

The effect of zinc on enzyme systems of *A. parasiticus* has been reported. The glycolytic enzymes were found to be zinc dependent *(92)* while the tricarboxylic acid cycle enzymes were not *(93)*. According to Niehaus and Dilts *(144,145)* zinc inhibits enzymes of the mannitol cycle and the pentose phosphate pathway. These authors proposed that the stimulatory effect of zinc on polyketide synthesis by *A. parasiticus* is mediated by inhibition of these enzymes causing a lowering of the cellular NADPH/NADP ratio and thus preventing conversion of malonyl coenzyme A to fatty acids.

Caffeine

Caffeine-containing commodities generally have a low incidence of aflatoxin contamination *(121,123)* even though *A. flavus* can be one of the predominant species associated with the mycoflora of these agricultural materials *(95,139)*. Further, inoculated samples of coffee and cocoa beans are poor substrates for aflatoxin production *(121,122,128,142,208)*. These observations led Buchanan and Fletcher *(38)* to evaluate caffeine for its effects on *A. parasiticus* cultured in microbiological media. They observed that both growth and toxin production were inhibited by caffeine, with the latter being more strongly affected. Subsequent studies with green and roasted coffee beans indicated that removal of caffeine greatly increased the potential for aflatoxin production *(142)*. Likewise, Lenovich *(121)* reported a strong correlation between the caffeine content of different varieties of cocoa beans and the ability of the varieties to resist aflatoxin production.

The inhibitory activity of caffeine appears to be highly specific in that other methylxanthines have little if any effect on the mold *(38,41,43)*. Buchanan et al. *(43)* hypothesized that inhibition of growth and aflatoxin production by caffeine may represent two separate effects since adenine and guanine partially overcame growth inhibition, but these compounds had little effect on inhibition of toxin formation. They also concluded that caffeine was not exerting its effect by acting as an inhibitor of cAMP phosphodiesterase. Using replacement cultures of *A. parasiticus,* Buchanan and Lewis *(39)* reported that caffeine inhibited respiration, glucose utilization and glucose transport, but did not affect the specific activities of various glycolytic enzymes. They hypothesized that caffeine was interfering with aflatoxin biosynthesis by depressing carbohydrate transport. However, recent studies (unpublished) in our laboratory with caffeine-resistant mutants of *A. parasiticus* have indicated that this hypothesis may need to be reevaluated since glucose transport in the mutant was caffeine-sensitive even though the strain was capable of producing abundant amounts of aflatoxins in the presence of caffeine.

A number of other mycotoxigenic species of the genera, *Aspergillus* and *Penicillium,* have been reported to be inhibited by caffeine *(26,42,44)*. Of particular interest is its effect on formation of another polyketide mycotoxin, ochratoxin A. Buchanan et al. *(44)* reported that

caffeine effectively inhibited toxin formation by *A. ochraceus* NRRL 3174. Tsubouchi et al. *(197)* reported that a number of strains of *A. ochraceus* isolated from green coffee beans were resistant to caffeine. These isolates only produced high levels of ochratoxin A when cultured in the presence of caffeine. Tsubouchi et al. *(197)* determined that caffeine-resistance in *A. ochraceus* was associated with the mold being capable of metabolizing caffeine. Buchanan et al. *(45)* recently reported the isolation of caffeine-resistant mutants of *A. parasiticus* that were only able to produce abundant amounts of aflatoxins when cultured in the presence of caffeine. However, in this instance neither the wild-type nor resistant strains were capable of metabolizing caffeine.

REFERENCES

1. Adye J., and R. I. Mateles. 1964. Incorporation of labelled compounds into aflatoxins. Biochim. Biophys. Acta 86:418-420.
2. Alderman G. G., and E. H. Marth. 1976. Inhibition of growth and aflatoxin production of *Aspergillus parasiticus* by citrus oils. Z. Lebensm. Unters. Forsch. 160:353-358.
3. Allcroft R., R. B. A. Carnaghan, K. Sargeant, and J. O'Kelly. 1961. A toxic factor in Brazilian groundnut meal. Vet. Rec. 73:428-429.
4. Alvarez-Barrea, V., A. M. Pearson, J. F. Price, J. I. Gray, and S. D. Aust. 1982. Some factors influencing aflatoxin production in fermented sausages. J. Food Sci. 47:1773-1775.
5. Anderson, M. S., and M. F. Dutton. 1979. The use of cell-free extracts derived from fungal protoplasts in the study of aflatoxin biosynthesis. Experientia 35:21-22.
6. Applebaum, R. S., and E. H. Marth. 1981. Biogenesis of the C_{20} polyketide, aflatoxin. A review. Mycopathologia 76:103-114.
7. Arseculeratne, S. N., U. Samarajeewa, and L. V. Welianga. 1976. Inhibition of aflatoxin accumulation in smoked substrates. J. Appl. Bacteriol. 41:223-233.
8. Asao, T., G. Büchi, M. M. Abdel-Kader, S. B. Chang, E. L. Wick, and G. N. Wogan. 1965. The structures of aflatoxins B_1 and G_1. J. Am. Chem. Soc. 87:882-886.
9. Asplin, F. D., and R. B. A. Carnaghan. 1961. The toxicity of certain groundnut meals for poultry with special reference to their effect on ducklings and chickens. Vet. Rec. 73:1215-1219.
10. Azzouz, M. A., and L. B. Bullerman. 1982. Comparative antimycotic effects of selected herbs, spices, plant components and commercial antifungal agents. J. Food Prot. 45:1298-1301.
11. Badii, F., M. O. Moss, and K. Wilson. 1986. The effect of sodium biselenite on the growth and aflatoxin production of *Aspergillus parasiticus* and the growth of other aspergilli. Lett. Appl. Microbiol. 2:61-64.
12. Bahk, J., and E. H. Marth. 1983. Growth and synthesis of aflatoxin by *Aspergillus parasiticus* in the presence of ginseng products. J. Food Prot. 46:210-215.
13. Bahk, J., and E. H. Marth. 1983. Aflatoxin production is inhibited by selected herbal drugs. Mycopathologia 83:129-134.
14. Bahk, J-R., I. Kwang-Sik, and J-K. Lee. 1985. Effects of crude saponin on growth and aflatoxin production by *Aspergillus parasiticus*. Korean J. Microbiol. 23:259-264. [Chem. Abstr. 104,105769b (1986)]
15. Basappa, S. C., V. Sreenivasamurthy, and H. A. B. Parpia. 1970. Aflatoxin and kojic acid production by resting cells of *Aspergillus flavus* Link. J. Gen. Microbiol. 6:81-86.
16. Batt, C., M. Solberg, and M. Ceponis. 1980. Inhibition of aflatoxin production by carrot root extract. J. Food Sci. 45:1210-1213.
17. Batt, C., M. Solberg, and M. Ceponis. 1983. Effect of volatile components of carrot seed oil on growth and aflatoxin production by *Aspergillus parasiticus*. J. Food Sci. 48:762-764,768.
18. Bean, G. A., and A. Rambo. 1975. Use of dimethyl sulfoxide to control aflatoxin production. Ann. N.Y. Acad. Sci. 243:238-245.
19. Bean, G. A., and A. Southall. 1983. Effect of pyridazinone herbicides on growth and aflatoxin release by *Aspergillus flavus* and *Aspergillus parasiticus*. Appl. Environ. Microbiol. 46:503-505.
20. Bennett, J. W., and S. B. Christensen. 1983. New perspectives on aflatoxin biosynthesis. Adv. Appl. Microbiol. 29:53-92.
21. Bennett, J. W., and L. S. Lee. 1979. Mycotoxins - their biosynthesis in fungi: Aflatoxins and other bisfuranoids. J. Food Prot. 42:805-809.
22. Bennett, J. W., L. S. Lee, and A. F. Cucullu. 1976. Effect of dichlorvos on aflatoxin and versicolorin A production in *Aspergillus parasiticus*. Bot. Gaz. (Chicago) 137:318-324.
23. Bennett, J. W., L. S. Lee, and G. G. Gaar. 1976. Effect of acetone on production of aflatoxins and versicolorin pigments by resting cell cultures of *Aspergillus parasiticus*. Mycopathologia 58:9-12.
24. Bennett, J. W., L. S. Lee, S. M. Shoss, and G. H. Boudreaux. 1980. Identification of averantin as an aflatoxin B_1 precursor: Placement in the biosynthetic pathway. Appl. Environ. Microbiol. 39:835-839.
25. Bennett, J. W., P. L. Rubin, L. S. Lee, and P. N. Chen. 1979. Influence of trace elements and nitrogen sources on versicolorin production by a mutant strain of *Aspergillus parasiticus*. Mycopathologia 69:161-166.
26. Betancourt, L. E., and H. K. Frank. 1983. Bedingungen des mikrobiellen Verderbs von grünem Kaffee. 2. Mitteilung: Bildung von Mykotoxinen. Dtsch. Lebensm. Rundsch. 79:404-407.
27. Beuchat, L. R. 1981. Influence of potassium sorbate and sodium benzoate on heat inactivation of *Aspergillus flavus, Penicillium puberulum* and *Geotrichum candidum*. J. Food Prot. 44:450-454.

27a. Bhatnager D., L. S. Lee, and R. H. Hill. 1986. Aflatoxin biosynthesis: Identification of o-methylsterigmatocystin as an aflatoxin B_1/G_1 precursor in *Aspergillus parasiticus*. Plant Physiol. 80 (Suppl.):18.

28. Bhatnagar, R. K., S. Ahmad, K. K. Kohli, K. G. Mukerji and T. A. Venkitasubramanian. 1982. Induction of polysubstrate monooxygenase and aflatoxin production by phenobarbitone in *Aspergillus parasiticus* NRRL 3240. Biochem. Biophys. Res. Commun. 104:1287-1292.
29. Bilgrami, K. S., R. S. Misra, K. K. Sinha, and P. L. Singh. 1979. Prevention of aflatoxin production in liquid and solid substrates through some plant extracts. Indian J. Exp. Biol. 17:1151.
30. Bilgrami, K. S., R. S. Misra, K. K. Sinha, and P. Singh. 1980. Effect of some wild and medicinal plant extracts on aflatoxin production and growth of *Aspergillus flavus* in liquid culture. J. Indian Bot. Soc. 59:123-126.
31. Bilgrami, K. S., K. K. Sinha, and P. Singh. 1981. Inhibition of aflatoxin production by ferulic acid on some cereals and oil seeds. Current Sci. 50:997-998.
32. Bilgrami, K. S., K. K. Sinha, and P. Singh. 1982. Prevention of aflatoxin production on some cereals and oil seeds by o-vanillin. Current Sci. 51:138.
33. Biollaz, M., G. Büchi, and G. Milne. 1968. Biosynthesis of aflatoxins. J. Am. Chem. Soc. 90:5017-5019.
34. Biollaz, M., G. Büchi, and G. Milne. 1970. The biosynthesis of the aflatoxins. J. Am. Chem. Soc. 92:1035-1043.
35. Blount, W. P. 1961. Turkey X disease. Turkeys. (J. Brit. Turkey Fed.) 9:52,55-58,77.
36. Brechbühler, S., G. Büchi, and G. Milne. 1967. The absolute configuration of the aflatoxins. J. Org. Chem. 32:2641-2642.
37. Buchanan, R. L., and J. C. Ayres. 1976. Effect of sodium acetate on growth and aflatoxin production by *Aspergillus parasiticus* NRRL 2999. J. Food Sci. 41:128-132.
38. Buchanan, R. L., and A. M. Fletcher. 1978. Methylxanthine inhibition of aflatoxin production. J. Food Sci. 43:654-655.
39. Buchanan, R. L., and D. F. Lewis. 1984. Caffeine inhibition of aflatoxin synthesis: probable site of action. Appl. Environ. Microbiol. 47:1216-1220.
40. Buchanan, R. L., and A. J. Shepherd. 1981. Inhibition of *Asper-*

gillus parasiticus by thymol. J. Food Sci. 46:976-977.
41. Buchanan, R. L., R. S. Applebaum, and P. Conway. 1978. Effect of theobromine on growth and aflatoxin production by *Aspergillus parasiticus*. J. Food Saf. 1:211-216.
42. Buchanan, R. L., M. A. Harry, and M. A. Gealt. 1983. Caffeine inhibition of sterigmatocystin, citrinin, and patulin production. J. Food Sci. 48:1226-1228.
43. Buchanan, R. L., D. G. Hoover, and S. B. Jones. 1983. Caffeine inhibition of aflatoxin production: mode of action. Appl. Environ. Microbiol. 46:1193-1200.
44. Buchanan, R. L., G. Tice, and D. Marino. 1982. Caffeine inhibition of ochratoxin A production. J. Food Sci. 47:319-321.
45. Buchanan, R. L., L. L. Zaika, C. A. Kunsch, C. J. Purcell, Jr., and S. E. Mertz. 1987. Isolation of a caffeine-resistant mutant of *Aspergillus parasiticus*. J. Food Sci. 52:194-196.
46. Büchi, G., D. M. Foulkes, M. Kuromo, G. F. Mitchell, and R. S. Schneider. 1967. The total synthesis of racemic aflatoxin B_1. J. Am. Chem. Soc. 89:6745-6753.
47. Bullerman, L. B. 1974. Inhibition of aflatoxin production by cinnamon. J. Food Sci. 39:1163-1165.
48. Bullerman, L. B. 1983. Effects of potassium sorbate on growth and aflatoxin production by *Aspergillus parasiticus* and *Aspergillus flavus*. J. Food Prot. 46:940-942.
49. Bullerman, L. B., F. Y. Lieu, and S. A. Seiler. 1977. Inhibition of growth and aflatoxin production by cinnamon and clove oils, cinnamic aldehyde and eugenol. J. Food Sci. 42:1107-1109,1116.
50. Chang, H. C., and A. L. Branen. 1975. Antimicrobial effects of butylated hydroxyanisole (BHA). J. Food Sci. 40:349-351.
51. Cheung, K. K., and G. A. Sim. 1964. Aflatoxin G_1: Direct determination of the structure by the method of isomorphous replacement. Nature (London) 201:1185-1188.
52. Chipley, J. R., L. D. Story, P. T. Todd, and J. J. Kabara. 1981. Inhibition of *Aspergillus* growth and extracellular aflatoxin accumulation by sorbic acid and derivatives of fatty acids. J. Food Saf. 2:109-120.
53. Chipley, J. R., and N. Uraih. 1980. Inhibition of *Aspergillus* growth and aflatoxin release by derivatives of benzoic acid. Appl. Environ. Microbiol. 40:352-357.
54. Cox, R. H., F. Churchill, R. J. Cole, and J. W. Dorner. 1977. Carbon-13 nuclear magnetic resonance studies of the structure and biosynthesis of versiconal acetate. J. Am. Chem. Soc. 99:3159-3161.
55. D'Agnolo, G., I. S. Rosenfeld, J. Awaya, S. Ōmura, and P. R. Vagelos. 1973. Inhibition of fatty acid synthesis by the antibiotic cerulenin. Specific inactivation of ß-ketoacyl-acyl carrier protein synthetase. Biochem. Biophys. Acta 326:155-166.
56. Davidson, P. M., and A. L. Branen. 1980. Antimicrobial mechanisms of butylated hydroxyanisole against two *Pseudomonas* species. J. Food Sci. 45:1607-1613.
57. Davidson, P. M., and A. L. Branen. 1981. Antimicrobial activity of non-halogenated phenolic compounds. J. Food Prot. 44:623-632.
58. Davis, N. D. 1981. Sterigmatocystin and other mycotoxins produced by *Aspergillus* species. J. Food Prot. 44:711-714.
59. Davis, N. D., and U. L. Diener. 1967. Inhibition of aflatoxin synthesis by p-aminobenzoic acid, potassium sulfite, and potassium flouride. App. Microbiol. 15:1517-1518.
60. Detroy, R. W., and C. W. Hesseltine. 1969. Net synthesis of ^{14}C-labeled lipids and aflatoxins in resting cells of *Aspergillus parasiticus*. Dev. Ind. Microbiol. 10:127-133.
61. Donkersloot, J. A., D. P. H. Hsieh, and R. I. Mateles. 1968. Incorporation of precursors into aflatoxin B_1. J. Am. Chem. Soc. 90:5020-5021.
62. Donkersloot, J. A., R. I. Mateles, and S. S. Yang. 1972. Isolation of averufin from a mutant of *Aspergillus parasiticus* impaired in aflatoxin biosynthesis. Biochem. Biophys. Res. Commun. 47:1051-1055.
63. Doyle, M. P., and E. H. Marth. 1979. Peroxidase activity in mycelia of *Aspergillus parasiticus* that degrade aflatoxin. Eur. J. Appl. Microbiol. 7:211-217.
64. Draughon, F. A. 1983. Control or suppression of aflatoxin production with pesticides. South Coop. Ser. Bull. 279:81-86.
65. Draughon, F. A., and J. C. Ayres. 1981. Inhibition of aflatoxin production by selected insecticides. Appl. Environ. Microbiol. 41:972-976.
66. Dutton, M. F. 1985. Characterization of metabolites from a strain of *Aspergillus flavus* accumulating aflatoxin B_2. S. Afr. J. Chem. 38:107-109.
67. Dutton, M. F., and M. S. Anderson. 1980. Inhibition of aflatoxin biosynthesis by organophosphorus compounds. J. Food Prot. 43:381-384.
68. Dutton, M. F., K. Ehrlich, and J. W. Bennett. 1985. Biosynthetic relationship among aflatoxins B_1, B_2, M_1 and M_2. Appl. Environ. Microbiol. 49:1392-1395.
69. Dutton, M. F., and J. G. Heathcote. 1966. Two new hydroxyaflatoxins. Biochem. J. 101:21P-22P.
70. Ehrlich, K., and A. Ciegler. 1984. Effect of phytate on aflatoxin formation by *Aspergillus parasiticus* and *Aspergillus flavus* in synthetic media. Mycopathologia 87:99-103.
71. Ehrlich, K., and A. Ciegler. 1985. Effect of phytate on aflatoxin formation by *Aspergillus* grown on different grains. Mycopathologia 92:3-6.
72. El-Gazzar, F. E., G. Rusul, and E. H. Marth. 1986. Growth and aflatoxin production by *Aspergillus parasiticus* in the presence of sodium chloride. J. Food Prot. 49:461-466.
73. Fabbri, A. A., C. Fanelli, G. Panfili, S. Passi, and P. Fasella. 1983. Lipoperoxidation and aflatoxin biosynthesis by *Aspergillus parasiticus* and *Aspergillus flavus*. J. Gen. Microbiol. 129:3447-3452.
74. Fabbri, A. A., G. Panfili, C. Fanelli, and A. Visconti. 1984. Effect of T-2 toxin on aflatoxin production. Trans. Br. Mycol. Soc. 83:150-152.
75. Failla, M. L. 1977. Zinc: Functions and transport in microorganisms. pp. 151-214. *In* E. D. Weinberg (ed.), Microorganisms and minerals. Marcel Dekker, Inc. New York.
76. Failla, L. J., and W. G. Niehaus, Jr. 1986. Regulation of Zn^{2+} uptake and versicolorin A synthesis in a mutant strain of *Aspergillus parasiticus*. Exp. Mycol. 10:35-41.
77. Fanelli, C., A. A. Fabbri, E. Finotti, P. Fasella, and S. Passi. 1984. Free radicals and aflatoxin biosynthesis. Experientia 40:191-193.
78. Fanelli, C., A. A. Fabbri, E. Finotti, and G. Panfili. 1983. Cerulenin and tetrahydrocerulenin: stimulating factors of aflatoxin biosynthesis. Trans. Br. Mycol. Soc. 81:201-204.
79. Fanelli, C., A. A. Fabbri, E. Finotti, and S. Passi. 1983. Stimulation of aflatoxin biosynthesis by lipophilic epoxides. J. Gen. Microbiol. 129:1721-1723.
80. Fanelli, C., A. A. Fabbri, and S. Passi. 1980. Growth requirements and lipid metabolism of *Aspergillus flavus*. Trans. Brit. Mycol. Soc. 75:371-375.
81. Fanelli, C., A. A. Fabbri, and S. Passi. 1981. Aflatoxin production by *Aspergillus flavus* during incubation with lipid sources in culture media. Trans. Br. Mycol. Soc. 77:416-419.
82. Fitzell, D. L., R. Singh, D. P. H. Hsieh, and E. L. Motell. 1977. Nuclear magnetic resonance identification of versiconal hemiacetal acetate as an intermediate in aflatoxin biosynthesis. J. Agric. Food Chem. 25:1193-1197.
83. Flanigan, B., and S. C. Hui. 1976. The occurrence of aflatoxin-producing strains of *Aspergillus flavus* in the mould floras of ground spices. J. Appl. Bacteriol. 41:411-418.
84. Floyd, J. C., J. W. Bennett, J. J. Dunn, and J. S. Fine. 1982. Growth of high aflatoxin B_2 mutants on defined and complex media and with ethoxyquin. Microbios 35:21-30.
85. Foudin, A. S., L. L. Leaich, and J. C. Ayres. 1978. Effects of ethoxyquin on aflatoxin-producing strains of *Aspergillus parasiticus* and *Aspergillus flavus*. J. Food Sci. 43:731-734.
86. Fung, D. Y. C., S. Taylor, and J. Kahan. 1977. Effects of butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) on growth and aflatoxin production of *Aspergillus flavus*. J. Food Saf. 1:39-51.

87. Gareis, M., J. Bauer, A. von Montgelas, and B. Gedek. 1984. Stimulation of aflatoxin B_1 and T-2 toxin production by sorbic acid. Appl. Environ. Microbiol. 47:416-418.
88. Ghosh, J., and P. Häggblom. 1985. Effect of sublethal concentrations of propionic or butyric acid on growth and aflatoxin production by *Aspergillus flavus*. Int. J. Food Microbiol. 2:323-330.
89. Goldblatt, L. A. (ed.). 1969. Aflatoxin. Academic press, New York.
90. Gueldner, R. C., D. M. Wilson, and A. R. Heidt. 1985. Volatile compounds inhibiting *Aspergillus flavus*. J. Agric. Food Chem. 33:411-413.
91. Gunasekaran, M. 1981. Optimum culture conditions for aflatoxin B_2 production by a human pathogenic strain of *Aspergillus flavus*. Mycologia 73:697-704.
92. Gupta, S. K., K. K. Maggon, and T. A. Venkitasubramanian. 1976. Zinc dependence of glycolytic enzymes of an aflatoxigenic strain of *Aspergillus parasiticus*. Microbios Lett. 3:89-92.
93. Gupta, S. K., K. K. Maggon, and T. A. Venkitasubramanian. 1977. Effect of zinc on tricarboxylic acid cycle intermediates and enzymes in relation to aflatoxin biosynthesis. J. Gen. Microbiol. 99:43-48.
94. Gupta, S. R., H. R. Prasanna, L. Wiswanathan, and T. A. Venkitasubramanian. 1975. The effect of inorganic salts and some biologically important compounds on the incorporation of [1-^{14}C]acetate into aflatoxins by resting mycelia of *Aspergillus parasiticus*. Z. Lebensm. Unters. Forsch. 157:19-22.
95. Hansen, A. P., and R. Welty. 1970. Microflora of raw cocoa beans. Mycopathol. Mycol. Appl. 44:309-316.
96. Harada, K., R. Higuchi, and I. Utsumi. 1968. Studies on sorbic acid. Part IV. Inhibition of the respiration in yeast. Agric. Biol. Chem. 32:940-946.
97. Hartley, R. D., B. F. Nesbitt, and J. O'Kelly. 1963. Toxic metabolites of *Aspergillus flavus*. Nature (London) 198:1056-1058.
98. Heathcote, J. G., and M. F. Dutton. 1969. New metabolites of *Aspergillus flavus*. Tetrahedron 25:1497-1500.
99. Heathcote, J. G., M. F. Dutton, and J. R. Hibbert. 1973. Biosynthesis of aflatoxins. Chem. Ind. (London) 1027-1030.
100. Heathcote, J. G., M. F. Dutton, and J. R. Hibbert. 1976. Biosynthesis of aflatoxins. Part II. Chem. Ind. (London) 270-272.
101. Heathcote, J. G., and J. R. Hibbert. 1978. Developments in food science 1. Aflatoxins: Chemical and biological aspects. Elsevier Sci. Publ. Co. Amsterdam.
102. Hitokoto, H., S. Morozumi, T. Wauke, S. Sakai, and H. Kurata. 1980. Inhibitory effects of spices on growth and toxin production of toxigenic fungi. Appl. Environ. Microbiol. 39:818-822.
103. Hitokoto, H., S. Morozumi, T. Wauke, S. Sakai and I. Ueno. 1978. Inhibitory effects of condiments and herbal drugs on the growth and toxin production of toxigenic fungi. Mycopathologia 66:161-167.
104. Holmquist, G. U., H. W. Walker, and H. M. Stahr. 1983. Influence of temperature, pH, water activity and antifungal agents on growth of *Aspergillus flavus* and *Aspergillus parasiticus*. J. Food Sci. 48:778-782.
105. Hsieh, D. P. H. 1973. Inhibition of aflatoxin biosynthesis of dichlorvos. J. Agric. Food Chem. 21:468-470.
106. Hsieh, D. P. H., and R. I. Mateles. 1970. The relative contribution of acetate and glucose to aflatoxin biosynthesis. Biochem. Biophys. Acta 208:482-486.
107. Hsieh, D. P. H., and R. I. Mateles. 1971. Preparation of labeled aflatoxins with high specific activities. Appl. Microbiol. 22:79-81.
108. Hsieh, D. P. H., J. N. Seiber, C. A. Reece, D. L. Fitzell, S. L. Yang, J. I. Dalezios, G. N. LaMar, D. L. Budd, and E. Motell. 1975. ^{13}C-nuclear magnetic resonance spectra of aflatoxin B_1 derived from acetate. Tetrahedron 31:661-663.
109. Jung, S-J., and M-J. Suh. 1985. Effects of steroidal carbamate derivatives on the aflatoxin productivity by *Aspergillus flavus*. Korean J. Mycol. 13:243-247.
110. Jurd, L., A. D. King, Jr., K. Mihara, and W. L. Stanley. 1971. Antimicrobial properties of natural phenols and related compounds. I. Obtusastyrene. Appl. Microbiol. 21:507-510.
111. Kachholz, T., and A. L. Demain. 1983. Nitrate repression of averufin and aflatoxin biosynthesis. J. Nat. Products (Lloydia) 46:499-506.
112. Karapinar, M. 1985. The effect of citrus oils and some spices on growth and aflatoxin production by *Aspergillus parasiticus* NRRL 2999. Int. J. Food Microbiol. 2:239-245.
113. Khan, S. N., K. K. Maggon, and T. A. Venkitasubramanian. 1978. Inhibition of aflatoxin biosynthesis by tolnaftate. Appl. Environ. Microbiol. 36:270-273.
114. King, Jr., A. D., A. D. Hocking, and J. I. Pitt. 1979. Dichloran-rose bengal medium for enumeration and isolation of molds from foods. Appl. Environ. Microbiol. 37:959-964.
115. Kobayashi, G. S., and G. Medoff. 1977. Antifungal agents, recent developments. Ann. Rev. Microbiol. 31:291-308.
116. Koreeda, M., B. Hulin, M. Yoshihara, C. A. Townsend, and S. B. Christensen. 1985. Synthesis and absolute configuration of (+)-averufin. J. Org. Chem. 50:5426-5428.
117. Lee, L. S., J. W. Bennett, A. F. Cucullu, and R. L. Ory. 1976. Biosynthesis of aflatoxin B_1: conversion of versicolorin A to aflatoxin B_1 by *Aspergillus parasiticus*. J. Agric. Food Chem. 24:1167-1170.
118. Lee, L. S., J. W. Bennett, A. F. Cucullu, and J. B. Stanley. 1975. Synthesis of versicolorin A by a mutant strain of *Aspergillus parasiticus* deficient in aflatoxin production. J. Agric. Food Chem. 23:1132-1134.
119. Lee, L. S., J. W. Bennett, L. A. Goldblatt, and R. E. Lundin. 1971. Norsolorinic acid from a mutant strain of *Aspergillus parasiticus*. J. Am. Oil Chem. Soc. 48:93-94.
120. Lee, E. G. H., P. M. Townsley, and C. C. Walden. 1966. Effect of bivalent metals on the production of aflatoxin in submerged cultures. J. Food Sci. 31:432-436.
121. Lenovich, L. M. 1981. Effect of caffeine on aflatoxin production on cocoa beans. J. Food Sci. 46:655,657.
122. Lenovich, L. M., and W. J. Hurst. 1979. Production of aflatoxin on cocoa beans. J. Assoc. Off. Anal. Chem. 62:1076-1079.
123. Levi, C. 1980. Mycotoxins in coffee. J. Assoc. Off. Anal. Chem. 63:1282-1285.
124. Liewen, M. B., and E. H. Marth. 1985. Growth and inhibition of microorganisms in the presence of sorbic acid: A review. J. Food Prot. 48:364-375.
125. Lin, C. C. S., and D. Y. C. Fung. 1983. Effect of BHA, BHT, TBHQ, and PG on growth and toxigenesis of selected aspergilli. J. Food Sci. 48:576-580.
126. Llewellyn, G. C., M. L. Burkett, and T. Eadie. 1981. Potential mold growth, aflatoxin production and antimycotic activity of selected natural spices and herbs. J. Assoc. Off. Anal. Chem. 64:955-960.
127. Llewellyn, G. C., E. C. Dixon, T. Eadie, W. V. Dashek and C. E. O'Rear. 1981. Aflatoxin formation on whole and ground cumin and anise seeds. J. Am. Oil Chem. Soc. 58:985A-988A.
128. Llewellyn, G. C., J. Benevides, and T. Eadie. 1978. Differential production of aflatoxin on natural and heat-treated cocoa beans. J. Food Prot. 41:785-787.
129. Mabrouk, S. S., and N. M. A. El-Shayeb. 1980. Inhibition of aflatoxin formation by some spices. Z. Lebensm. Unters. Forsch. 171:344-347.
130. Mabrouk, S. S., N. M. A. El-Shayeb, A. H. El-Refai, L. A. R. Sallam, and A. A. Hamby. 1985. Inhibitory activities of some marine algae on aflatoxin accumulation. Appl. Microbiol. Biotechnol. 22:152-155.
131. Madhyastha, M. S., and R. V. Bhat. 1984. *Aspergillus parasiticus* growth and aflatoxin production on black and white pepper and the inhibitory action of their chemical constituents. Appl. Environ. Microbiol. 48:376-379.
132. Maggon, K. K., S. K. Gupta, and T. A. Venkitasubramanian. 1977. Biosynthesis of aflatoxins. Bacteriol. Rev. 41:822-855.
133. Maggon, K. K., and T. A. Venkitasubramanian. 1973. Metabolism of aflatoxins B_1 and G_1 by *Aspergillus parasiticus*. Experientia 29:1210-1211.

134. Mallozzi, M. A. B., C. A. Campacci, and S. Chiba. 1985. Acao do acido propionico na producao de aflatoxina e no crescimento de *Aspergillus flavus*. Summa Phytopathol. 11:220-229.
135. Marsh, P. B., M. E. Simpson, and M. W. Trucksess. 1975. Effects of trace metals on the production of aflatoxins by *Aspergillus parasiticus*. Appl. Microbiol. 30:52-57.
136. Marshall, D. L., and L. B. Bullerman. 1986. Effect of sucrose esters in combination with selected mold inhibitors on growth and aflatoxin production by *Aspergillus parasiticus*. J. Food Prot. 49:378-382.
137. Mateles, R. I., and J. C. Adye. 1965. Production of aflatoxins in submerged culture. Appl. Microbiol. 13:208-211.
138. Mayura, K., S. C. Basappa, and V. Sreenivasa Murthy. 1985. Studies on some factors affecting aflatoxin production by *Aspergillus flavus* and *A. parasiticus*. J. Food Sci. Technol. 22:126-129.
138a. McCormick, S. P., D. Bhatnagar, and L. S. Lee. 1987. Averufanin is an aflatoxin B_1 precursor between averantin and averufin in the biosynthetic pathway. Appl. Environ. Microbiol. 53:14-16.
139. Mislivec, P. B., V. R. Bruce, and R. Gibson. 1983. Incidence of toxigenic and other molds in green coffee beans. J. Food Prot. 46:969-973.
140. Morozumi, S. 1978. Isolation, purification and antibiotic activity of o-methoxycinnamaldehyde from cinnamon. Appl. Environ. Microbiol. 36:577-583.
141. Moss, M. O., and F. Badii. 1982. Increased production of aflatoxins by *Aspergillus parasiticus* Speare in the presence of rubratoxin B. Appl. Environ. Microbiol. 43:895-898.
142. Nartowicz, V. B., R. L. Buchanan, and S. Segall. 1979. Aflatoxin production in regular and decaffeinated coffee beans. J. Food Sci. 44:446-448.
143. Nesbitt, B. F., J. O'Kelly, K. Sargeant, and A. Sheridan. 1962. Toxic metabolites of *Aspergillus flavus*. Nature (London) 195:1062-1063.
144. Niehaus, W. G., Jr., and R. P. Dilts, Jr. 1982. Purification and characterization of mannitol dehydrogenase from *Aspergillus parasiticus*. J. Bacteriol. 151:243-250.
145. Niehaus, W. G., Jr., and R. P. Dilts, Jr. 1984. Purification and characterization of glucose-6-phosphate dehydrogenase from *Aspergillus parasiticus*. Arch. Biochem. Biophys. 228:113-119.
146. Niehaus, W. G., Jr., and L. J. Failla. 1984. Effect of zinc on versicolorin production by a mutant strain of *Aspergillus parasiticus*. Exp. Mycol. 8:80-84.
147. Nomura, S., T. Horiuchi, S. Omura, and T. Hata. 1972. The action mechanism of cerulenin. I. Effect of cerulenin on sterol and fatty acid biosynthesis in yeast. J. Biochem (Tokyo) 71:783-796.
148. Obioha, W. I., H. M. Stahr, and A. A. Kraft. 1983. Effect of sodium nitrate on aflatoxin production in pork sausage at different temperatures and the effect of nitrite on growth of *Aspergillus parasiticus* in culture. J. Agric. Food Chem. 31:1039-1042.
149. O'Malley, G. J., R. A. Murphy, and M. P. Cava. 1985. Aflatoxin precursors: Total synthesis of (±)-averufin and (±)-nidurufin. J. Org. Chem. 50:5533-5537.
150. Pachler, K. G. R., P. S. Steyn, R. Vleggaar, and P. L. Wessels. 1976. Carbon-13 nuclear magnetic resonance assignments and biosynthesis of aflatoxin B_1 and sterigmatocystin. J. Chem. Soc. Perkin Trans. I., 1182-1189.
151. Passi, S., C. Fanelli, A. A. Fabbri, E. Finotti, G. Panfili, and M. Nazzaro-Porro. 1985. Effect of halomethanes on aflatoxin induction in cultures of *Aspergillus parasiticus*. J. Gen Microbiol. 131:687-691.
152. Passi, S., M. Nazzaro-Porro, C. Fanelli, A. A. Fabbri, and P. Fasella. 1984. Role of lipoperoxidation in aflatoxin production. Appl. Microbiol. Biotechnol. 19:186-190.
153. Prasad, T. 1983. Precursors and biogenetic schemes of aflatoxin synthesis. A review. Proc. Symp. Mycotoxin in Food and Feed, Bhagalpur, pp. 173-197.
154. Przybylski, K. S., and L. B. Bullerman. 1980. Influence of sorbic acid on viability and ATP content of conidia of *Aspergillus parasiticus*. J. Food Sci. 45:375-376,385.
155. Pupovac-Velikonja, A., J. Velikonja, and A. Dürrigl. 1986. Kultur von *Aspergillus parasiticus* im Apfelsaft. I. Einfluss von Natriumbenzoat und Kaliumsorbat auf Pilzwachstum und Aflatoxin-Biosynthese. Z. Lebensm.Unters. Forsch. 182:303-306.
156. Rabie, C. J., C. J. Meyer, L. van Heerden, and A. Lübben. 1981. Inhibitory effect of molybdenum and vanadium salts on aflatoxin B_1 synthesis by *Aspergillus flavus*. Can. J. Microbiol. 27:962-967.
157. Raj, H. G., L. Viswanathan, H. S. R. Murthy, and T. A. Venkitasubramanian. 1969. Biosynthesis of aflatoxins by cell-free preparations from *Aspergillus flavus*. Experientia 25:1141-1142.
158. Rama Devi, G., and H. Polasa. 1984. Inhibition of growth and toxin production by lauric acid derivatives in *Aspergillus* species. Current Sci. 53:811-813.
159. Rama Devi, G., and H. Polasa. 1985. Diacetyl - a growth and toxin inhibitor of aspergilli. J. Stored Prod. Res. 21:195-197.
160. Rao, H. R. G., and P. K. Harein. 1972. Dichlorvos as an inhibitor of aflatoxin production on wheat, corn, rice and peanuts. J. Econ. Entomol. 65:988-989.
161. Ray, L. L., and L. B. Bullerman. 1982. Preventing growth of potentially toxic molds using antifungal agents. J. Food Prot. 45:953-963.
162. Recknagel, R. O., E. A. Glende, Jr., and A. M. Hruszkewych. 1977. Chemical mechanisms in carbontetrachloride toxicity. pp. 97-132. *In* A. A. Pryor, (ed.), Free radicals in biology. Vol. 3. Academic Press, New York.
163. Reiss, J. 1982. Development of *Aspergillus parasiticus* and formation of aflatoxin B_1 under the influence of conidiogenesis affecting compounds. Arch. Microbiol. 133:236-238.
164. Ruprich, J., and A. Piskac. 1985. Ucinek pripravku Kurasan (ethoxyquin) na toxinogenni vlastnosti plisne *Aspergillus flavus*. Vet. Med. (Prague) 30:239-246.
165. Salomao, R. de Cassia, and A. Purchio. 1982. Atividade de substancias de origem vegetal na inhibicão do crescimento e na producão de aflatoxinas por *Aspergillus flavus* e *A. parasiticus*. Rev. Microbiol., São Paulo 13:394-401.
166. Sargeant, K., A. Sheridan, J. O'Kelly, and R. B. A. Carnaghan. 1961. Toxicity associated with certain samples of ground nuts. Nature (London) 192:1095-1097.
167. Schroeder, H. W., and W. W. Carlton. 1973. Accumulation of only aflatoxin B_2 by a strain of *Aspergillus flavus*. Appl. Microbiol. 25:146-148.
168. Schroeder, H. W., R. J. Cole, R. D. Grigsby, and H. Hein, Jr. 1974. Inhibition of aflatoxin production and tentative indentification of an aflatoxin intermediate "versiconal acetate" from treatment with dichlorvos. Appl. Microbiol. 27:394-399.
169. Schroeder, H. W., and W. H. Kelton. 1975. Production of sterigmatocystin by some species of the genus *Aspergillus* and its toxicity to chicken embryos. Appl. Microbiol. 30:589-591.
170. Seenappa, M., and A. G. Kempton. 1980. *Aspergillus* growth and aflatoxin production on black pepper. Mycopathologia 70:135-137.
171. Sharma, A., S. R. Padwal-Desai, and G. B. Nadkarni. 1985. Possible implication of reciprocity between ethylene and aflatoxin biogenesis in *Aspergillus flavus* and *Aspergillus parasiticus*. Appl. Environ. Microbiol. 49:79-82.
172. Sharma, A., G. M. Tewari, A. J. Shrikhande, S. R. Padwal-Desai, and C. Bandyopadhyay. 1979. Inhibition of aflatoxin-producing fungi by onion extracts. J. Food Sci. 44:1545-1547.
173. Shih, C.-N., and E. H. Marth. 1974. Aflatoxin formation, lipid synthesis and glucose metabolism by *Aspergillus parasiticus* during incubation with and without agitation. Biochim. Biophys. Acta 338:286-296.
174. Simpson, T. J., A. E. de Jesus, P. S. Steyn, and R. Vleggaar. 1982. Biosynthesis of aflatoxins. Incorporation of [2-$^2H_3$]acetate and [1-^{13}C, 2-$^2H_3$]acetate into averufin. J. Chem. Soc. Chem. Commun. 632-634.
175. Simpson, T. J., A. E. de Jesus, P. S. Steyn, and R. Vleggaar. 1982. Biosynthesis of aflatoxins. Incorporation of [4'-$^2H_2$]averufin into aflatoxin B_1 by *Aspergillus flavus*. J. Chem. Soc. Chem. Commun. 631-632.

176. Simpson, T. J., A. E. de Jesus, P. S. Steyn, and R. Vleggaar. 1983. Biosynthesis of aflatoxins. Incorporation of [2-$^{2}H_{3}$]acetate into aflatoxin B_1 by *Aspergillus flavus*. J. Chem. Soc. Chem. Commun. 338-340.
177. Singh, R., and D. P. H. Hsieh. 1976. Enzymatic conversion of sterigmatocystin into aflatoxin B_1 by cell-free extracts of *Aspergillus parasiticus*. Appl. Environ. Microbiol. 31:743-745.
178. Singh, R., and D. P. H. Hsieh. 1977. Aflatoxin biosynthetic pathway: Elucidation by using blocked mutants of *Aspergillus parasiticus*. Arch. Biochem. Biophys. 178:285-292.
179. Sinha, K. K., and P. Singh. 1981. Effect of some phenolics on aflatoxin production and growth of *Aspergillus parasiticus*. Indian Phytopathol. 34:530-531.
180. Sofos, J. N., and F. F. Busta. 1981. Antimicrobiol activity of sorbate. J. Food Prot. 44:614-622.
181. Stewart, R. G., R. D. Wyatt, and M. D. Ashmore. 1977. The effect of various antifungal agents on aflatoxin production and growth characteristics of *Aspergillus parasiticus* and *Aspergillus flavus* in liquid medium. Poultry Sci. 56:1630-1635.
182. Steyn, P. S., R. Vleggaar, P. L. Wessels and D. B. Scott. 1975. Biosynthesis of aflatoxin B_1 from [2-^{13}C]- and [1,2-^{13}C]-acetate. J. Chem. Soc. Chem. Commun. 193-195.
183. Steyn, P. S., R. Vleggaar, and P. L. Wessels. 1980. The biosynthesis of aflatoxin and its congeners. pp. 105-155. *In* P. S. Steyn (ed.), The biosynthesis of mycotoxins. A study in secondary metabolism. Academic Press, New York.
184. Stoloff, L. 1977. Aflatoxins - An overview. pp. 7-28. *In* J. V. Rodricks, C. W. Hesseltine and M. A. Mehlman (eds.), Mycotoxins in human and animal health. Pathotox. Publishers, Inc. Park Forrest South, Illinois.
185. Stoloff, L. 1980. Aflatoxin M in perspective. J. Food Prot. 43:226-230.
186. Swaminathan, B., and P. E. Koehler. 1976. Isolation of an inhibitor of *Aspergillus parasiticus* from white potatoes (*Solanum tuberosum*). J. Food Sci. 41:313-319.
187. Tiwari, R. P., V. Mittal, T. C. Bhalla, S. S. Saini, G. Singh, and D. V. Vadehra. 1986. Effect of metal ions on aflatoxin production by *Aspergillus parasiticus*. Folia Microbiol. 31:124-128.
188. Tiwari, R. P., V. Mittal, G. Singh, T. C. Bhalla, S. S. Saini, and D. V. Vadehra. 1986. Effect of fatty acids on aflatoxin production by *Aspergillus parasiticus*. Folia Microbiol. 31:120-123.
189. Townsend, C. A., and S. B. Christensen. 1983. Stable isotope studies of anthraquinone intermediates in the aflatoxin pathway. Tetrahedron 39:3575-3582.
190. Townsend, C. A., and S. B. Christensen. 1985. Concerning the role of nidurufin in aflatoxin biosynthesis. J. Am. Chem. Soc. 107:270-271.
191. Townsend, C. A., and S. B. Christensen. 1986. Stereochemical correlation of (-)-averantin. Tetrahedron Lett. 27:887-888.
192. Townsend, C. A., and S. G. Davis. 1983. The regiochemistry of A-ring-labelled averufin incorporation into aflatoxin B_1. J. Chem. Soc. Chem. Commun. 1420-1422.
193. Townsend, C. A., S. B. Christensen, and S. G. Davis. 1982. Bisfuran formation in aflatoxin biosynthesis: The fate of the averufin side chain. J. Am. Chem. Soc. 104:6152-6153.
194. Townsend, C. A., S. B. Christensen, and K. Trautwein. 1984. Hexanoate as a starter unit in polyketide biosynthesis. J. Am. Chem. Soc. 106:3868-3869.
195. Townsend, C. A., S. G. Davis, M. Koreeda, and B. Hulin. 1985. A cationic model of the chain-branching step in aflatoxin biosynthesis. J. Org. Chem. 50:5428-5430.
196. Tsai, W-Y. J., K-P. P. Shao, and L. B. Bullerman. 1984. Effects of sorbate and propionate on growth and aflatoxin production of sublethally injured *Aspergillus parasiticus*. J. Food Sci. 49:86-90.
197. Tsubouchi, H., H. Terada, K. Yamamoto, K. Hisada, and Y. Sakabe. 1985. Caffeine degradation and increased ochratoxin A production by toxigenic strains of *Aspergillus ochraceus* isolated from green coffee beans. Mycopathologia 90:181-184.
198. Turner, R. B., D. L. Lindsey, D. D. Davis, and R. D. Bishop. 1975. Isolation and identification of 5,7-dimethoxyisoflavone, an inhibitor of *Aspergillus flavus* from peanuts. Mycopathologia 57:39-40.
199. Tyagi, J. S., A. K. Tyagi, and T. A. Venkitasubramanian. 1981. Some factors regulating [1-^{14}C]acetate incorporation into aflatoxins by spheroplasts and spheroplast lysates from *Aspergillus parasiticus*. Toxicon 19:445-454.
200. Uraih, N., T. R. Cassity, and J. R. Chipley. 1977. Partial characterization of the mode of action of benzoic acid on aflatoxin biosynthesis. Can. J. Microbiol. 23:1580-1584.
201. Uraih, N., and J. R. Chipley. 1976. Effects of various acids and salts on growth and aflatoxin production by *Aspergillus flavus* NRRL 3145. Microbios 17:51-59.
202. Uraih, N., and L. Ogbadu. 1982. Influence of woodsmoke on aflatoxin production by *Aspergillus flavus*. Eur. J. Appl. Microbiol. Biotechnol. 14:51-53.
203. Valcarcel, R., J. W. Bennett, and J. Vitanza. 1986. Effect of selected inhibitors on growth, pigmentation, and aflatoxin production by *Aspergillus parasiticus*. Mycopathologia 94:7-10.
204. Van Soest, T. C., and A. F. Peerdeman. 1970. Crystal structure of aflatoxin B_1. I. Structure of the chloroform solvate of aflatoxin B_1 and the absolute configuration of aflatoxin B_1. Acta Crystallogr. Sect. B. 26:1940-1947.
205. Van Soest, T. C., and A. F. Peerdeman. 1970. Crystal structure of aflatoxin B_2. Acta Crystallogr. Sect. B. 26:1956-1963.
206. Vederas, J. C., and T. T. Nakashima. 1980. Biosynthesis of averufin by *Aspergillus parasiticus;* detection of ^{18}0-label by ^{13}C-N.M.R. isotope shifts. J. Chem. Soc. Chem. Commun. 183-185.
207. Venkitasubramanian, T. A. 1977. Biosynthesis of aflatoxin and its control. pp. 83-98. *In* J. V. Rodricks, C. W. Hesseltine and M. A. Mehlman (eds.), Mycotoxins in human and animal health. Pathotox Publishers, Inc. Park Forrest South, Illinois.
208. Wildman, J. D., L. Stoloff, and R. Jacobs. 1967. Aflatoxin production by a potent *Aspergillus flavus* Link isolate. Biotechnol. Bioeng. 9:429-437.
209. Wilson, D. M., R. C. Gueldner, J. K. McKinney, R. H. Lievsay, B. D. Evans, and R. A. Hill. 1981. Effects of β-ionone on *Aspergillus flavus* and *Aspergillus parasiticus* growth, sporulation, morphology and aflatoxin production. J. Am. Oil Chem. Soc. 58:959A-961A.
210. Wotton, H. R., and R. N. Strange. 1985. Circumstantial evidence for phytoalexin involvement in the resistance of peanuts to *Aspergillus flavus*. J. Gen. Microbiol. 131:487-494.
211. Yao, R. C., and D. P. H. Hsieh. 1974. Step of Dichlorvos inhibition in the pathway of aflatoxin biosynthesis. Appl. Microbiol. 28:52-57.
212. York, G. K., and R. H. Vaughn. 1964. Mechanisms in the inhibition of microorganisms by sorbic acid. J. Bacteriol. 88:411-417.
213. Yousef, A. E., S. M. El-Gendy, and E. H. Marth. 1980. Growth and biosynthesis of aflatoxin by *Aspergillus parasiticus* in cultures containing nisin. Z. Lebensm. Unters. Forsch. 171:341-343.
214. Yousef, A. E., and E. H. Marth. 1981. Growth and synthesis of aflatoxin by *Aspergillus parasiticus* in the presence of sorbic acid. J. Food Prot. 44:736-741.
215. Yousef, A. E., and E. H. Marth. 1983. Incorporation of [^{14}C]acetate into aflatoxin by resting cultures of *Aspergillus parasiticus* in the presence of antifungal agents. Eur. J. Appl. Microbiol. Biotechnol. 18:103-108.
216. Yousef, A. E., and E. H. Marth. 1984. Kinetics and accumulation of aflatoxin B_1 by *Aspergillus parasiticus* in the presence of butylated hydroxyanisole, isoprothiolane and nystatin. Biotechnol. Bioeng. 26:6-11.